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PHYTOPATHOLOGY

VOLUME 19 NUMBER 2

FEBRUARY, 1929

THE PEACH APHID (MYZUS PERSICAE SULZ.) AS AN AGENT IN VIRUS TRANSMISSION[1]

ISMÉ A. HOGGAN[2]

INTRODUCTION

Following the report by Allard (1) of the transmission of tobacco mosaic by aphids, increasing attention has been paid to the relation of insects to plant virus diseases. Numerous investigations in recent years have demonstrated the ability of various species of aphids and leafhoppers especially to transmit certain viruses. Apart from the peculiar scientific interest involved in these relationships, considerable importance has been attached to such insects as agents in virus dissemination, and the rôle played by them in disease epidemics has been strongly emphasized. The peach aphid (*Myzus persicae* Sulz.), in particular, has been reported as capable of transmitting several apparently distinct diseases of this group (5, 11, 15, 16, 17, 18, 19).

As a result of several years' work at the Wisconsin Agricultural Experiment Station on the overwintering and spread of tobacco mosaic in the field, the importance of aphids in the dissemination of this particular disease has, however, been questioned (13). A detailed investigation of this phase of the problem has consequently been undertaken. While the work naturally involves a consideration of more than one species of aphid in relation to virus transmission, the present paper is limited to a report of certain greenhouse studies of a single species only, namely, the green peach aphid, *Myzus persicae* Sulz. This aphid occurs commonly upon tobacco and other plants in the greenhouse, and is one of two species previously reported by Allard (3) as active carriers of tobacco mosaic.

[1] Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Office of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

[2] I wish to acknowledge gratefully my indebtedness to Dr. James Johnson, at whose suggestion this work was undertaken, for his advice and encouragement throughout the course of the investigation; to Dr. S. P. Doolittle and Dr. C. F. Lackey for furnishing specimens of *Myzus persicae;* and to Dr. A. A. Granovsky for verifying the identification of the species.

MATERIALS AND METHODS

Since a large number of solanaceous plant species in addition to tobacco are known to be susceptible to tobacco mosaic (1), and since some of these have been reported as wild hosts of the virus (1, 9), it seemed desirable to include several other species beside tobacco in a study of the transmission of this mosaic. For the sake of comparison, it was decided to investigate also the transmission of a second mosaic disease between the same host species in parallel trials. Cucumber mosaic has been shown to be transferable to a number of solanaceous host plants by the method of artificial juice inoculation (8, 12); this disease was selected for comparative study.

In the actual choice of plant species, it was therefore necessary to consider two factors: (1) susceptibility of the species to both viruses, and (2) suitability as a food plant for the peach aphid. The following six species were finally selected: tobacco (*Nicotiana tabacum*), *N. rustica, N. glauca, N. glutinosa,* pepper (*Capsicum annuum*), and ground cherry (*Physalis pubescens*). These plants are all more or less easily infected with either virus by methods of artificial inoculation, and the peach aphid feeds and multiplies readily upon them all. Furthermore, with two exceptions, both viruses are readily recoverable from infected plants by artificial means. In the case of *N. glutinosa,* however, it has been shown by Allard (1) and by Johnson (12) that, although tobacco mosaic may have a marked effect upon this host, the plant juices are apparently not infectious. On pepper, too, although cucumber mosaic produces typical mosaic symptoms, yet the virus is rarely, and with difficulty, recoverable from this host by artificial means; in other words, pepper is a poor "source of inoculum" (14) for the cucumber mosaic virus. These two hosts were included, however, with a view to determining whether or not they would behave as equally poor sources of inoculum in respect to aphid transmission.

The two viruses were supplied by Dr. James Johnson, that of tobacco mosaic [*Tobacco virus 1* (14)] coming originally from a diseased tobacco plant on the University grounds at Madison and that of cucumber mosaic [*Cucumber virus 1* (14)] from a mosaic cucumber plant furnished by Dr. S. P. Doolittle, United States Department of Agriculture. Both were maintained by repeated transfer on tobacco in the greenhouse.

Specimens of the peach aphid were taken from some tobacco plants in the greenhouse and the identification verified by Dr. A. A. Granovsky of the Wisconsin Experiment Station. These aphids proved to be non-viruliferous. Subsequent colonies reared upon cabbage in an insect-proof cage furnished a source of non-viruliferous aphids which was employed throughout the investigation. The aphids appeared to thrive particularly well upon this host and multiplied with great rapidity on it. The colonies con-

sisted of the "summer generations" or yellowish-green, viviparous females, the majority apterous and a low, somewhat variable, proportion winged. So far as could be determined, no other forms of this aphid were present at any time. Since cabbage is not susceptible to either tobacco or cucumber mosaic, there was little danger of these colonies becoming infected with either virus. Nevertheless, their condition in this respect was determined from time to time by transferring a number of individuals to healthy, young tobacco plants. All such tobacco plants remained healthy, indicating that the aphids did not carry any transmissible virus.

In studying the transmission of the two viruses, the different hosts were infected with each mosaic disease and large numbers of aphids colonized upon them in separate cages. After five to eight or more days, the aphids were transferred to healthy plants of the species required for investigation. In all transmission experiments, only young, vigorously growing plants were used, and these were handled in sets of five. The aphids were transferred from the mosaic host by removing one or more of the aphid-bearing leaves and laying them on the healthy plant. A piece of paper was usually placed under such leaves to prevent any contact with the healthy plant. About 30–40 or more adult aphids, together with a large number of nymphs, were transferred in this manner to each plant; these soon migrated to the new host, and the mosaic leaf and paper were subsequently removed with sterile forceps. The aphids fed and multiplied abundantly upon the new hosts in all cases. Usually parallel transfers were made simultaneously for each virus. In each trial, an equal number of plants was treated in a similar manner as a control, the mosaic leaves in this case being, however, entirely free from aphids. The plants were placed after treatment in a large glass chamber divided into insect-proof sections, the infested plants and the controls being kept in different sections. The temperature of the chamber normally ran from 75° to 80° F., but occasionally varied from about 70° to 90° F. After about a week, the plants were brought out into a warm greenhouse (80°–90° F.), which was immediately fumigated. This greenhouse was fumigated regularly twice a week to keep it free from aphids, and no infested plants were brought into it except immediately before fumigation. The plants were discarded after two to three weeks, since any mosaic symptoms appearing after this time would not be accepted as resulting from primary aphid infection.

EXPERIMENTAL RESULTS

It is not believed necessary to enter into a full description of the symptoms produced by the two viruses on the different host plants employed. The chief differential symptoms have been described by Johnson (14), and

the reader is referred to this author for further details. It should be emphasized, however, that the two diseases are readily distinguishable upon all six hosts when young plants are used. Generally speaking, cucumber mosaic on these host plants is characterized by marked chlorosis, malformation, and mottling; tobacco mosaic by marked stunting and (tobacco and *N. glauca* excepted) by a progressive necrosis of the plants. In any case of doubt as to diagnosis, juice inoculations were made to tobacco, when the virus could be readily identified.

The results of the comparative transmission experiments with *Myzus persicae* are summarized in table 1. It will be seen from this table that transmission of the cucumber mosaic virus by the aphid was readily secured among all six hosts, 100 per cent infection commonly occurring in different trials. Of a total of 370 plants tested, 345 became infected with cucumber mosaic, as compared with 8 out of 370 plants in the control series (Plate I, B, C, E, F, H, J and Plate II, B, C, E, F, H, J). Aphid transmission of this virus was also obtained to tomato (*Lycopersicon esculentum*) from tobacco, *N. rustica*, and tomato, and to cucumber (*Cucumis sativus*) from tobacco and pepper. In the latter transfers, it was interesting to note that although the aphids attempted to feed upon the young cucumber plants, they did not stay on this host for any length of time, and after 48 hours all had died or disappeared. Nevertheless, 100 per cent infection was obtained in each trial, all control plants remaining healthy.

It will be observed that the percentage of infection resulting in the different trials varied very little with the species of host employed, whether this served as a source of the virus or as a host to which the virus was transmitted. Pepper in particular, though a very poor source of inoculum for cucumber mosaic virus as far as artificial juice inoculation is concerned, proved to be as suitable a source of inoculum with respect to aphid transmission as any of the five other hosts employed. Again, while these hosts have been found to differ considerably in relative susceptibility to infection with cucumber mosaic through artificial means, they appear to be all about equally susceptible to infection by aphids. For example, while it is comparatively easy to obtain 100 per cent infection of tobacco with cucumber mosaic by methods of artificial inoculation, this has rarely or never been obtained by the writer on certain other hosts such as pepper, *N. glauca*, and *N. glutinosa*. Comparable results have been obtained by Johnson (14). On the other hand, equally high percentages of infection resulted from aphid infection on all hosts studied.

Infection by artificial means was most readily secured on these less susceptible hosts by repeatedly pricking viruliferous juice into the midribs

Mosaic host (source of virus)	Mosaic virus	Hosts												Total infections	
		N. tabacum		*N. glauca*		*N. glutinosa*		*N. rustica*		*C. annuum*		*P. pubescens*			
		Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
Nicotiana tabacum	Cucumber	30/26	30/1	10/9	10/0	10/10	10/0	15/15	15/0	15/15	15/0	15/14	15/0	95/89	95/1
	Tobacco	30/0	30/0	10/0	10/0	10/0	10/0	20/1	20/1	15/0	15/0	15/0	15/0	100/1	100/1
N. glauca	Cucumber	15/13	15/0	10/10	10/0	5/3	5/0	10/10	10/0	10/9	10/0	10/10	10/1	60/55	60/1
	Tobacco	15/0	15/0	10/0	10/0	5/0	5/0	10/0	10/0	10/0	10/0	10/0	10/0	60/0	60/0
N. glutinosa	Cucumber	5/4	5/0	5/4	5/0	5/5	5/0	10/9	10/1	5/5	5/0	5/4	5/0	35/31	35/1
	Tobacco	5/0	5/0	5/0	5/0	5/0	5/0	10/0	10/1	5/0	5/0	5/0	5/0	35/0	35/1
N. rustica	Cucumber	15/12	15/0	10/9	10/0	5/5	5/0	10/10	10/2	10/10	10/0	15/15	15/0	65/61	65/2
	Tobacco	15/0	15/0	10/0	10/0	5/0	5/0	10/0	10/0	10/0	10/0	10/0	10/0	60/0	60/0
Capsicum annuum	Cucumber	10/8	10/0	10/10	10/0	5/5	5/0	10/10	10/1	10/8	10/0	10/8	10/0	55/49	55/1
	Tobacco	10/0	10/0	10/0	10/0	5/0	5/0	10/0	10/0	10/0	10/0	10/0	10/0	55/0	55/0
Physalis pubescens	Cucumber	15/15	15/1	10/10	10/0	5/5	5/0	10/10	10/0	10/10	10/0	10/10	10/1	60/60	60/2
	Tobacco	20/2	20/0	10/0	10/0	5/0	5/0	30/6[b]	30/1	10/0	10/0	10/1	10/0	85/9	85/1
Total infections	Cucumber	90/78	90/2	55/52	55/0	35/33	35/0	65/64	65/4	60/57	60/0	65/61	65/2	370/345	370/8
	Tobacco	95/2	95/0	55/0	55/0	35/0	35/0	90/7	90/3	60/0	60/0	60/1	60/0	395/10	395/3

[a] 'Infested' denotes those plants to which aphids were transferred from a mosaic host; 'control' denotes those plants which were treated with mosaic leaves free from aphids and which served as controls to the infested plants. In all fractions, the numerator represents the total number of plants either infested with aphids or treated as controls, as the case may be; the denominator the number of such plants which developed symptoms of mosaic.

[b] Four of these infections probably resulted from handling of the plants (see text).

of the leaves; the incubation period was then relatively short and about the same as that resulting from aphid infection, the minimum observed in each case being six days. When viruliferous juice was pricked or scratched only into the lamina of the leaves, however, an extremely low percentage of infection resulted, together with a somewhat longer incubation period. Since the aphids appeared to feed usually upon the larger veins of the leaf, this may in part account for the higher percentages of infection produced by them. In any case, it is evident that the peach aphid is a highly efficient agent of transmission of cucumber mosaic among certain solanaceous host plants and, generally speaking, a more reliable means of securing infection than present methods of artificial inoculation. Doolittle (7) reports a similar situation in respect to the transmission of the same mosaic disease by the melon aphid (*Aphis gossypii* Glover).

Contrary to previous expectation, no evidence was obtained from these experiments of the transmission of tobacco mosaic by the peach aphid among any of the host plants employed (Plate I, A, D, G and Plate II, A, D, G). Of a total of 395 plants tested, only 10 developed symptoms of the disease, as compared with 3 out of 395 plants in the controls (table 1). These few cases of infection are believed to arise from certain sources other than aphid transmission, probably from contact with infectious materials of different kinds. In one of the earlier trials of the transmission of tobacco mosaic from *Physalis* to *N. rustica*, the infested plants were handled on one occasion with unsterilized forceps and four of the five plants subsequently became mosaic. Frequent repetitions of this transfer, conducted with the utmost care, resulted in only a single case of mosaic in 20 plants; hence the preceding infections were not regarded as an indication of aphid transmission. Tobacco mosaic is known to be extremely infectious through various means of contact, and *N. rustica* appeared to be even more susceptible than tobacco in this respect. The incident quoted above serves to illustrate this point. It will be noted that 10 of the 13 cases of tobacco mosaic developing on both infested and control plants occurred upon *N. rustica*. Occasional infections were noted also on the plants employed in the cucumber mosaic trials. In any case, the percentage of infection obtained in the trials with tobacco mosaic was exceedingly low (2.6 per cent) and of the same order as that occurring in the controls (0.8 per cent). When compared with the percentage of infection obtained with cucumber mosaic (93 per cent), it is evident that the results should not be interpreted as indicative of any transmission by aphids of the tobacco mosaic virus.

It is not believed that the failure of the aphid to transmit this virus is due to any fault in technique, on account of the ready transmission of the cucumber mosaic virus obtained by the same methods and under identical

conditions. Nor is it believed to be due to the use of insufficient numbers of aphids, since, in various trials, 60 or more adults, together with a large number of nymphs, were transferred to each host plant without any infection resulting, while, on the other hand, preliminary experiments have indicated that as few as five adults alone may be sufficient to cause infection with cucumber mosaic. In regard to the duration of feeding on the mosaic host, in some cases the colonies remained on such hosts for as long as two weeks before transference to healthy plants, and again no infection resulted.

Although Allard (1) has shown that the tobacco mosaic virus is distributed throughout the vegetative parts of infected plants, there remained a possibility that the virus was not present, in sufficient quantity to cause infection, in the actual leaves upon which the aphids had been feeding while on the mosaic hosts. In order to examine this possibility, in a number of trials selected at random, juice inoculations were made to tobacco from each aphid-bearing leaf transferred from the mosaic to the healthy host, as soon as the aphids had migrated from it. The results, which are shown in table 2, clearly demonstrate that in all cases the virus was present in these leaves in sufficient quantity to produce infection through artificial inoculation, although no aphid transmission took place.

Two other strains of *Myzus persicae* have been tested in relation to the transmission of the two viruses and similar results have been obtained with each. One of these strains, taken on sugar beet at Riverside, California, was received from Dr. C. F. Lackey, U. S. Department of Agriculture; the other, taken on tomato at Bradenton, Florida, from Dr. S. P. Doolittle. While only limited trials were conducted in these cases, the results secured are sufficiently decisive to indicate that strains of the peach aphid from different localities may be expected to behave alike in respect to the transmission of the two viruses under consideration. These results are given in table 3.

More conclusive evidence, perhaps, was furnished by the following experiment. It seemed reasonable to assume that if the peach aphid is incapable of transmitting the tobacco mosaic virus, then colonies, reared upon plants infected simultaneously with both tobacco and cucumber mosaic, should transmit only the cucumber mosaic virus from the combination. An experiment designed to test this hypothesis has in fact demonstrated a definite selectivity in virus transmission by this aphid. Aphids were colonized upon young tobacco plants which had been artificially inoculated with both viruses and which showed definite symptoms of the combination disease. After seven days, the aphids were transferred to healthy tobacco plants by the usual leaf method and an equal number of plants were treated as controls with mosaic leaves free from aphids. When

TABLE 2.—*A comparison of the transmission of tobacco mosaic by Myzus persicae with artificial inoculation from the leaves on which the aphids were feeding when transferred from the mosaic host*[a]

Aphid transmission			Artificial inoculation		
Mosaic host (source of virus)	Host to which aphids transferred	Results	Mosaic host (source of virus)	Host inoculated with juice from aphid-bearing leaves[b]	Results
Nicotiana tabacum	*Physalis pubescens*	5/0	*N. tabacum*	*N. tabacum*	5/5
do	*N. rustica*	10/1	do	do	10/10
do	*N. tabacum*	10/0	do	do	10/10
N. glauca	*N. rustica*	5/0	*N. glauca*	do	5/5
Capsicum annuum	*C. annuum*	5/0	*C. annuum*	do	5/5
do	*N. tabacum*	5/0	do	do	5/5

[a] In all fractions, the numerator represents the total number of plants either infested with aphids or artificially inoculated, as the case may be; the denominator the number of such plants which developed symptoms of mosaic.

[b] The "aphid-bearing leaves" are those on which the aphids were feeding when transferred from the mosaic to the healthy hosts.

TABLE 3.—*Comparative trials of the transmission of the cucumber and tobacco mosaic viruses by strains of Myzus persicae from different localities*[a]

TABLE 3.—*Comparative trials of the transmission of the cucumber and tobacco mosaic viruses by strains of Myzus persicae from different localities*[a]

Source of aphids	Mosaic host (source of virus)	Mosaic virus	Hosts						Total infections	
			N. tabacum		*C. annuum*		*N. glauca*			
			Infested	Control	Infested	Control	Infested	Control	Infested	Control
Bradenton, Fla.	*Nicotiana tabacum*	Cucumber	10/10	10/0					10/10	10/0
		Tobacco	10/0	10/0					10/0	10/0
	Capsicum annuum	Cucumber	5/5	5/0	5/5	5/0			10/10	10/0
		Tobacco	5/0	5/0	5/0	5/0			10/0	10/0
	Total infections	Cucumber							20/20	20/0
		Tobacco							20/0	20/0
Riverside, Cal.	*N. tabacum*	Cucumber	5/4	5/0			5/4	5/0	10/8	10/0
		Tobacco	5/0	5/0			5/0	5/0	10/0	10/0
	C. annuum	Cucumber	5/5	5/0	5/5	5/0			10/10	10/0
		Tobacco	5/0	5/0	5/0	5/0			10/0	10/0
	Physalis pubescens	Cucumber	5/5	5/0					5/5	5/0
		Tobacco	5/0	5/0					5/0	5/0
	Total infections	Cucumber							25/23	25/0
		Tobacco							25/0	25/0

[a] 'Infested' denotes those plants to which aphids were transferred from a mosaic host; 'control' denotes those plants which were treated with mosaic leaves free from aphids and which served as controls to the infested plants. In all fractions, the numerator represents the total number of plants either infested with aphids or treated as controls, as the case may be; the denominator the number of such plants which developed symptoms of mosaic.

the aphids had migrated to the new hosts, the mosaic leaves upon which they had previously been feeding were ground up separately in small quantities of sterile water and the liquid allowed to stand for 48 hours in sterilized test-tubes in order to destroy any cucumber mosaic virus present. The contents of each tube were then inoculated to tobacco to determine the presence of any tobacco mosaic virus in the extract. The experiment was repeated three times and similar results were obtained in each trial (table 4). Of a total of 20 aphid-infested plants, 18 developed typical

TABLE 4.—*Selective virus transmission by Myzus persicae from tobacco plants infected simultaneously with both tobacco and cucumber mosaic*

Tobacco plant to which aphids were transferred from mosaic host[a]	Viruses transmitted by aphids	Results of artificial inoculation to tobacco from aphid-bearing leaf[b]	Selective transmission of cucumber mosaic virus by aphids[c]
No. 1	Cucumber mosaic virus	+	+
2	None	+	-
3	Cucumber mosaic virus	+	+
4	do	-	-
5	do	+	+
6	do	+	+
7	do	+	+
8	do	-	-
9	do	+	+
10	do	+	+
11	do	+	+
12	None	+	-
13	Cucumber mosaic virus	+	+
14	do	+	+
15	do	+	+
16	do	+	+
17	do	+	+
18	do	+	+
19	do	+	+
20	do	+	+

[a] As controls to these aphid-infested plants, 20 tobacco plants were treated with mosaic leaves free from aphids; all remained healthy.

[b] The "aphid-bearing leaf" is the leaf on which the aphids were feeding when transferred from the mosaic host. + denotes infection with tobacco mosaic obtained; - denotes no infection.

[c] + denotes selective transmission demonstrated; - denotes selective transmission not demonstrated.

cucumber mosaic only and 2 remained healthy. None showed any symptoms of tobacco mosaic. Since cucumber mosaic is readily distinguishable from the combination disease, there could be no doubt as to the results secured. All control plants remained healthy. Of the 18 aphid-bearing leaves transferred from the mosaic hosts to these 18 subsequently infected plants, 16 were shown by artificial inoculation to tobacco to contain the tobacco mosaic virus. In 16 out of 20 cases, the occurrence of selective transmission by the aphid was thus definitely established (Fig. 1). It is evident that, although both viruses may be present in the leaves upon which this aphid feeds, the aphid is capable of transmitting only the cucumber mosaic virus from the combination. Similar cases of selective transmission by aphids have been reported, as, for example, that of *Aphis maidis* Fitch, which readily transmits grass mosaic from infected to healthy corn (6), but which apparently does not transmit streak disease to the same host (20).

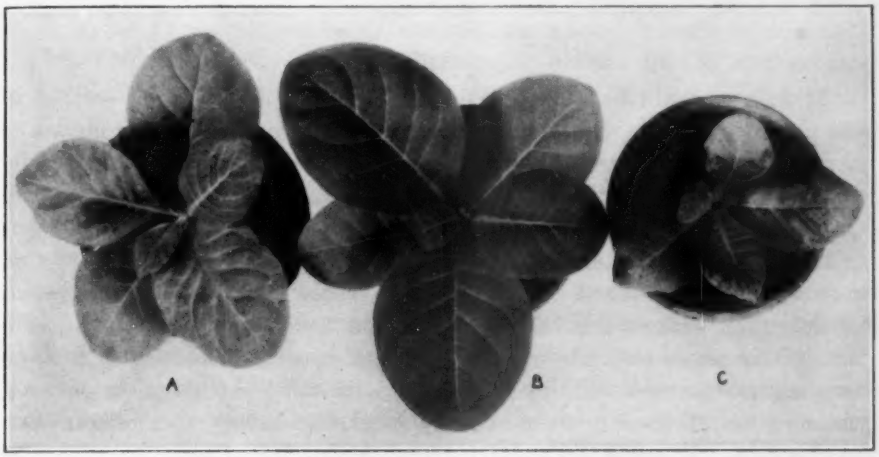

FIG. 1. Illustrating selective virus transmission by *Myzus persicae* Sulz. from tobacco plants infected simultaneously with tobacco and cucumber mosaic.

A. Tobacco plant infested with aphids from tobacco infected with the combination disease. This plant became infected with cucumber mosaic only.

B. Tobacco plant treated as a control to A with a leaf, free from aphids, from tobacco infected with the combination disease. This plant remained healthy.

C. Tobacco plant artificially inoculated with juice extracted from the leaf on which the aphids were feeding when transferred from the mosaic host to plant A. The juice was aged *in vitro* for two days before inoculation. This plant became infected with tobacco mosaic only.

Note the difference in symptoms between cucumber mosaic and tobacco mosaic on tobacco, as illustrated by A and C. In the greenhouse, cucumber mosaic on this host is usually characterized by chlorosis of the whole or part of the leaf, with little or no true mottling or savoying. Tobacco mosaic is usually characterized by a marked stunting of the plant, with distinct mottling and savoying of the leaf.

These results are somewhat surprising, perhaps, in view of the fact that tobacco mosaic is, ordinarily speaking, much the more highly infectious disease of the two among susceptible solanaceous plants. Any slight injury to the epidermis which results in the introduction of a minute quantity of virus into the tissues may be sufficient to cause infection of the plant with tobacco mosaic. The results therefore suggest that there may be something more involved in aphid transmission of viruses than a mere mechanical transfer of viruliferous juice from plant to plant. In this connection it may be appropriate to mention that the peach aphid has been reported as an agent in the transmission of several other plant virus diseases, namely: spinach blight (15), potato mosaic (19), potato leaf roll (5, 18), lettuce mosaic (11), sugar beet mosaic (16), and mosaic of Chinese cabbage, mustard and turnip (17). If these diseases are all due to different specific viruses, then the failure of the peach aphid to transmit the tobacco mosaic virus may have an added significance.

DISCUSSION

It will no doubt be necessary first to correlate the results described in the preceding pages with those reported by Allard (1, 3), who claims to have obtained ready transmission of tobacco mosaic in the greenhouse by the peach aphid. While there is a possibility of this investigator having employed a strain of *Myzus persicae* which differed radically in its behavior in relation to virus transmission, this possibility is considered unlikely on account of the consistent results which have been obtained in the present investigation with strains of this aphid from three widely different localities. It is suggested, however, that Allard may not, in fact, have been securing transmission of ordinary tobacco mosaic, but actually, perhaps, transmission of some other mosaic on tobacco, possibly cucumber mosaic on this host. It seems likely that cucumber mosaic had been introduced at one time or another into his greenhouse. The "specific mosaic disease in *Nicotiana viscosum*" (=*N. glutinosa*), reported by him (2), agrees very closely in description with what we now know as cucumber mosaic on this host, and the symptoms illustrated by him (2, Plate 35) are suggestive of typical infection with this disease. It is possible also that the mosaic diseases of *Phytolacca decandra* (4) and pepper (2), reported by him, may have been cucumber mosaic on these hosts. It should be remembered that, under certain conditions, the symptoms of the two diseases on tobacco may be very similar and difficult to distinguish from one another; that the knowledge of specificity in mosaic disease was at that time relatively limited; and that cucumber mosaic, as such, had not yet been adequately described or recognized in scientific literature. It is therefore tentatively

suggested that, while Allard was essentially correct in his report of the transmission of a virus disease of tobacco by the peach aphid, yet that disease was not ordinary tobacco mosaic, but cucumber mosaic on tobacco.

The results described in this paper have a certain significance in relation to the dissemination and control of ordinary tobacco mosaic in the field. Although all experiments have been conducted in the greenhouse, there seems no obvious reason to expect any radical difference in the behavior of the peach aphid, with respect to virus transmission, under other environmental conditions. Again, while only one form of this insect has been studied, namely, the alate or apterous viviparous female which constitutes the "summer generations," the various other forms apparently do not develop under natural conditions during most, if not all, of the time that tobacco is in the field (10) and on this account would not merit serious consideration from the practical standpoint as possible agents in the dissemination of the disease. While it is probable that certain other species of aphid may be capable of transmitting tobacco mosaic in the field, it appears exceedingly doubtful whether the peach aphid is responsible for the dissemination of this particular disease.

SUMMARY

1. In extensive greenhouse trials, it has been demonstrated that the green peach aphid (*Myzus persicae* Sulz.) readily transmits the cucumber mosaic virus between tobacco and certain other susceptible solanaceous host plants.

2. As compared with methods of artificial inoculation, the peach aphid behaves as a highly efficient agent in the transmission of this virus.

3. Comparative trials, carried out under identical conditions, gave no evidence of transmission of the tobacco mosaic virus by the same aphid between the same host species.

4. The peach aphid exhibits a definite selectivity in virus transmission. Colonies reared upon tobacco plants, infected with both cucumber and tobacco mosaic, transmit only the cucumber mosaic virus from the combination, although the tobacco mosaic virus is present in the leaves upon which the aphids have fed.

5. This selectivity in virus transmission suggests that the relation between aphid and virus may not be purely mechanical.

6. It is not believed that the peach aphid is responsible for the dissemination of ordinary tobacco mosaic in the field.

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EXPLANATION OF PLATES

PLATE I

Illustrating the results secured in comparative trials of the transmission of the cucumber and tobacco mosaic viruses by *Myzus persicae* Sulz.

A. Tobacco infested with aphids from *Physalis pubescens* infected with tobacco mosaic. This plant remained healthy.

C. Tobacco infested with aphids from *P. pubescens* infected with cucumber mosaic. This plant became infected with cucumber mosaic.

B. Tobacco treated as a control to C with a leaf, free from aphids, from *P. pubescens* infected with cucumber mosaic. This plant remained healthy.

D. *Nicotiana rustica* infested with aphids from *N. glauca* infected with tobacco mosaic. This plant remained healthy.

F. *N. rustica* infested with aphids from *N. glauca* infected with cucumber mosaic. This plant became infected with cucumber mosaic.

E. *N. rustica* treated as a control to F with a leaf, free from aphids, from *N. glauca* infected with cucumber mosaic. This plant remained healthy.

G. *Physalis pubescens* infested with aphids from *Nicotiana glauca* infected with tobacco mosaic. This plant remained healthy.

J. *P. pubescens* infested with aphids from *N. glauca* infected with cucumber mosaic. This plant became infected with cucumber mosaic.

H. *P. pubescens* treated as a control to J with a leaf, free from aphids, from *N. glauca* infected with cucumber mosaic. This plant remained healthy.

PLATE II

Illustrating the results secured in comparative trials of the transmission of the cucumber and tobacco mosaic viruses by *Myzus persicae* Sulz.

A. Pepper infested with aphids from tobacco infected with tobacco mosaic. This plant remained healthy.

C. Pepper infested with aphids from tobacco infected with cucumber mosaic. This plant became infected with cucumber mosaic.

B. Pepper treated as a control to C with a leaf, free from aphids, from tobacco infected with cucumber mosaic. This plant remained healthy.

D. *Nicotiana glauca* infested with aphids from *N. rustica* infected with tobacco mosaic. This plant remained healthy.

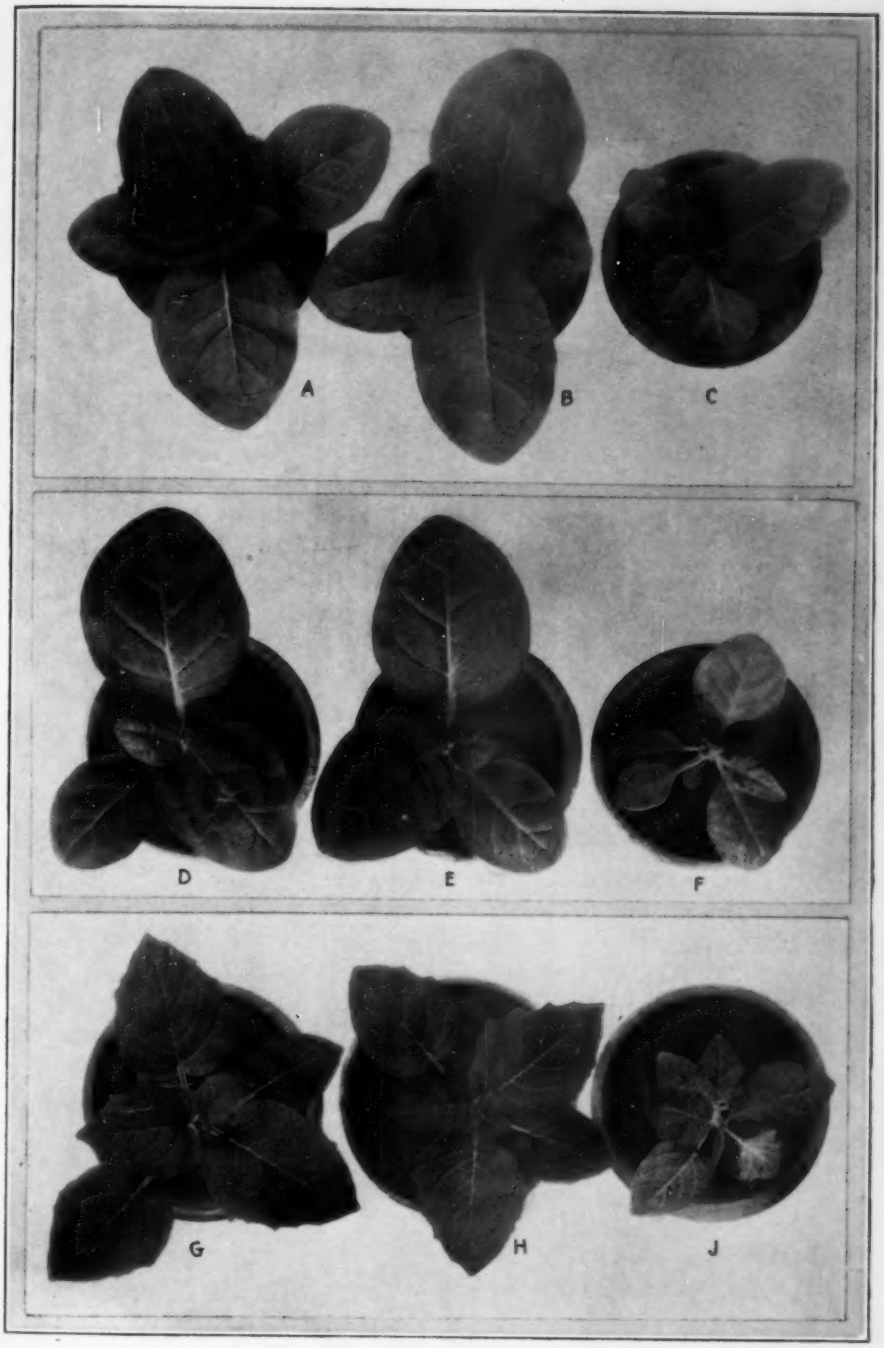
F. *N. glauca* infested with aphids from *N. rustica* infected with cucumber mosaic. This plant became infected with cucumber mosaic.

E. *N. glauca* treated as a control to F with a leaf, free from aphids, from *N. rustica* infected with cucumber mosaic. This plant remained healthy.

G. *Nicotiana glutinosa* infested with aphids from pepper infected with tobacco mosaic. This plant remained healthy.

J. *N. glutinosa* infested with aphids from pepper infected with cucumber mosaic. This plant became infected with cucumber mosaic.

H. *N. glutinosa* treated as a control to J with a leaf, free from aphids, from pepper infected with cucumber mosaic. This plant remained healthy.



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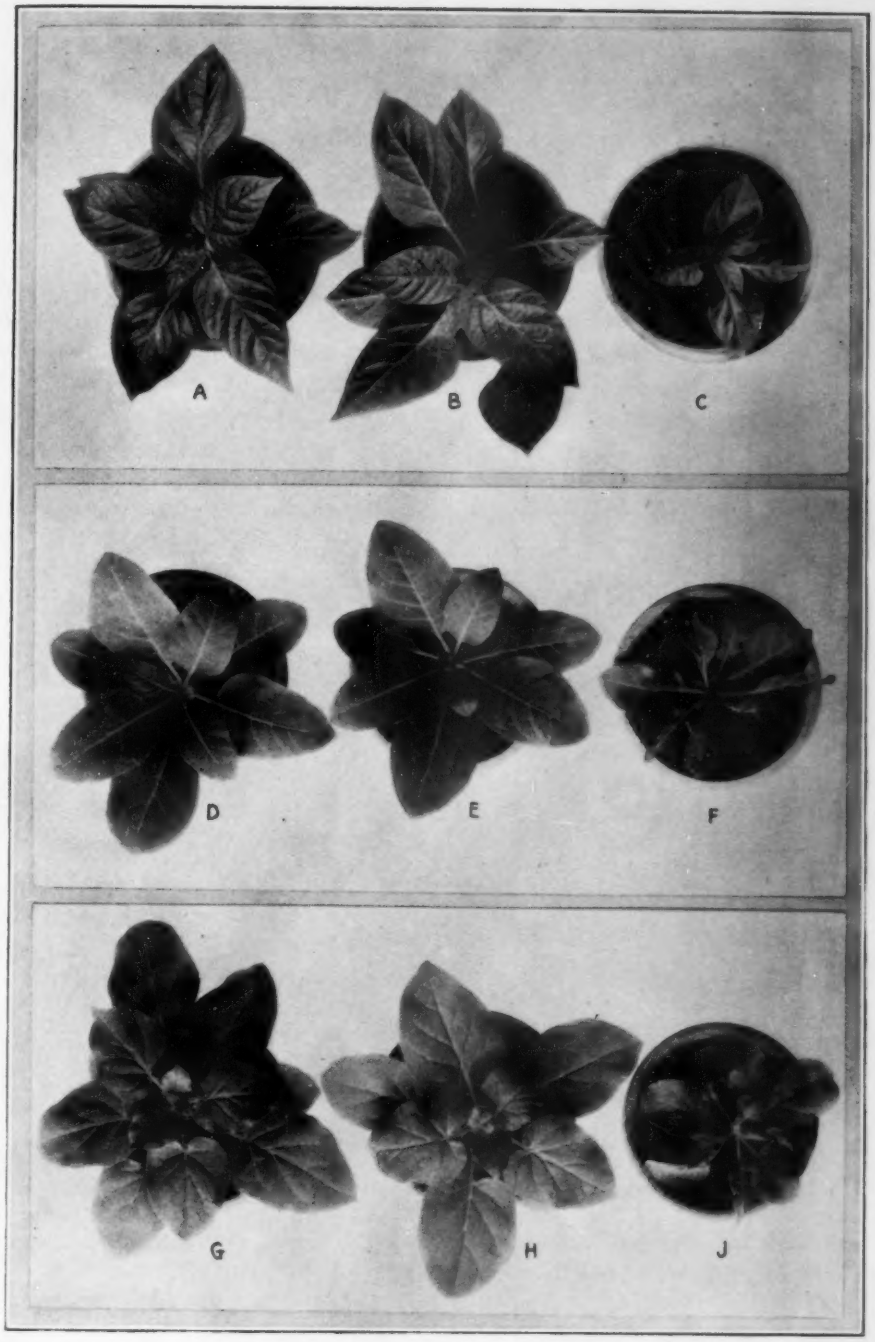

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WHITE-SPOT OF ALFALFA AND ITS RELATION TO IRRIGATION[1]

B. L. RICHARDS[2]

INTRODUCTION

White-spot was first described as a specific disease of alfalfa by Stewart, French, and Wilson (7) in 1908. Since that date various other workers have called attention to the disease, indicating that it is widespread throughout the United States, although of little economic importance. As reported by O'Gara (4) in 1918, the problem under irrigated conditions is somewhat different. Not infrequently in Utah the disease occurs throughout a field and injures the crop.

The factors responsible for white-spot have remained obscure, and in various reports on the disease but little has been contributed to the under-

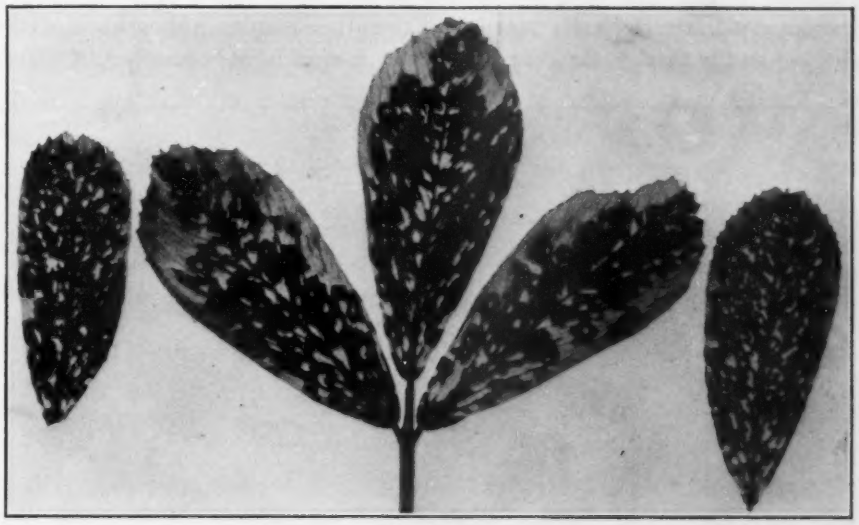

FIG. 1.—White-spot of alfalfa. Leaflets showing spot type lesion, and an entire leaf showing spot and marginal injury.

[1] Contribution from Department of Botany and Plant Pathology, Utah Agricultural Experiment Station. Approved for publication by Director, April 17, 1928.

[2] Botanist and Plant Pathologist. The writer wishes to express his appreciation to Dr. F. B. Wann and H. Loran Blood, of the Botany Department of the Utah Agricultural Experiment Station.

standing of its real nature. In fact, the true cause of the disease as it commonly occurs in the field remains unknown.

In May, 1926, white-spot occurred in such abundance in northern Utah as to cause considerable concern among farmers and to warrant detailed study by the plant pathological division of the Utah Experiment Station. This study was continued through the summers of 1926 and 1927 and has yielded such data with respect to the nature and cause of white-spot as to justify this preliminary report.

SYMPTOMS AND THE IDENTITY OF WHITE-SPOT IN VARIOUS REGIONS

White-spot is characterized generally by writers as small, definitely delimited and bleached necrotic areas in the leaf mesophyll. (See Figs. 1 and 2.) All leaves on a stem, and ordinarily all leaves on all stems of a single crown, become involved simultaneously and usually more or less equally affected. The spots are variable in shape, with abrupt margins. Very little if any spread of the lesion is evident. The clear and uniform appearance of the tissues within the lesions provides little suggestion of a parasitic origin. In fact, the tissues in the early stages of development, though dead, are perfectly sterile. The rather sudden appearance of the disease in the field is striking, and when a considerable number of plants

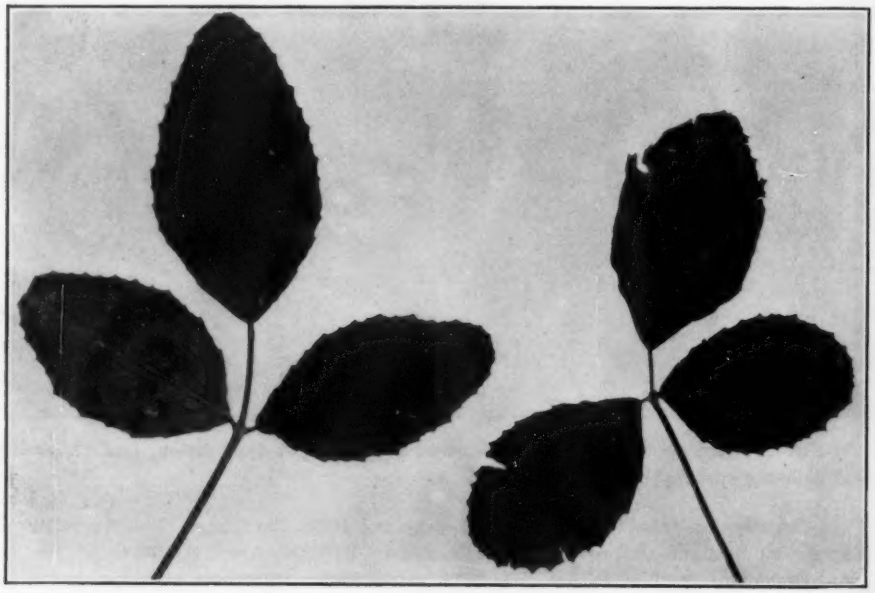

FIG. 2.—White-spot as it appears on sweet clover (*Melilotus alba*). Left, early stage of lesion development. Right, final stage of development.

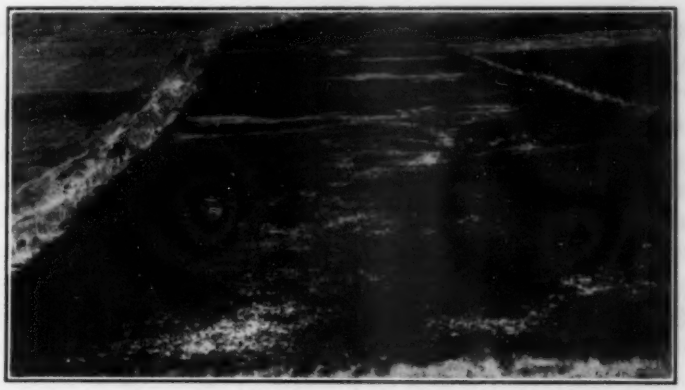

FIG. 3.—Mr. Williamson's field at Farmville in 1926. Irrigated during a heavy rain on May 4, first observed May 9, and photographed May 10. The severely diseased patches, which appear white, developed only in areas of vigorous plant growth. In such patches, 80–100 per cent of the plants were affected.

in an area become affected they may be distinguished from a considerable distance (Figs. 3 and 4).

Since the etiology of white-spot is so elusive, the question naturally arises as to whether the disease as observed by the early workers throughout the United States is the same as that observed so frequently in Utah during 1926 and 1927. Descriptions, photographs, and personal observations, however, confirm rather than question the identity.

FIG. 4.—Three alfalfa fields in the Farmville district in 1927. At left, Mr. Williamson's field studied in 1926 (Cf. Fig. 3). Water was started on his field at 6 A. M., May 13; on the center field at noon; and on the field at right at 5 P. M. It was allowed to run on all three until 6 A. M., May 14. Photographed May 16, 48 hours later than photograph shown in figure 5.

O'Gara (4) states that he has observed white-spot throughout the Middle Western, Intermountain, and Pacific Coast States; and further states that he has submitted white-spot which was artificially produced in his plots to several plant pathologists, who reported that his specimens were identical with white-spot which they themselves had collected. W. W. Jones (3) identified the disease occurring in 1926 and that produced experimentally by the present writer in the fields of Cache Valley as identical with disease observed previously by O'Gara and him in Utah and other western states. In addition, Jones states (3) that "In the spring of 1917 specimens taken in Salt Lake Valley were sent to F. C. Stewart and C. H. Crabill for comparison with the disease specimens observed in New York and Virginia. Both of these observers affirmed an identity of condition."

During October of 1926 the writer submitted specimens of white-spot produced experimentally in Cache Valley to Dr. F. R. Jones, who recognized the disease as being of the same nature if not identical with that which he had observed in alfalfa fields throughout the United States.

Through the courtesy of Dr. C. G. Coons, in 1926 the writer procured specimens of white-spot collected in Michigan in 1915. Additional fresh specimens were sent from Michigan in 1927 by Professor Ray Nelson. When compared, these specimens from Michigan could not be distinguished from those collected in Utah during the various studies throughout the summers of 1926 and 1927.

Whether or not such peculiar spotting of alfalfa is produced by one or a number of factors, it is quite evident that the lesions appearing in various parts of the United States are so similar as to be indistinguishable.

DISTRIBUTION AND ECONOMIC IMPORTANCE OF WHITE-SPOT

From published reports white-spot is known to occur in New York State (7), Connecticut (1), and Virginia (2, 5), as well as throughout Middle Western, Pacific Coast and Intermountain States (4). In addition, through personal conferences the writer has learned of its occurrence in Michigan, Wisconsin, Illinois, Indiana, and Ohio. It is quite possible that the disease occurs to a limited extent wherever alfalfa is grown.

In general, white-spot is considered of minor economic importance. In speaking of the disease in New York, Stewart (7) states: "The disease occurs in alfalfa fields all over the state, but we have never seen it sufficiently abundant to cause appreciable loss." Clinton (1) observes that the spots are confined to scattered plants in the field and that it is "not a very important or injurious trouble." Similar reports have been given to the writer by various pathologists, although there are others who maintain that the disease is worthy of more than passing attention and that it is

of far greater importance than is usually attributed to it. O'Gara (4) reports that "usually the disease is not considered serious by alfalfa growers, but in many instances the writer has noted that the disease may be more or less disastrous and may produce a very decided loss in yield." A similar view is held by W. W. Jones (3), who has observed the disease in Utah for a number of years.

In the survey conducted during May, 1926, white-spot was discovered in a considerable number of fields in each of the following counties: Salt Lake, Utah, Davis, Weber, Boxelder, and Cache. In Salt Lake, Utah, and Cache Counties the disease was especially severe. For example, in Cache Valley, 98 affected fields were noted along the main road from Wellsville to Richmond, a distance of approximately 20 miles. Several of these fields were seriously damaged, varying in percentage of plants injured from 15 to 75. In the most severely injured fields, a crop loss of as high as 20–35 per cent resulted. This loss consisted in retarded growth, leaf mesophyll destruction, leaf drop, and frequently, in severe cases, the killing of the entire plant.

The following year (1927) the disease appeared in serious form only in local areas, especially in Salt Lake and Cache Counties. In such local areas, because of its annual occurrence, white-spot must be considered as an important factor in alfalfa production.

OBJECTIVES AND METHODS OF STUDY

Studies were undertaken with the prime objective of determining the nature and cause of white-spot, as well as to ascertain more accurately its distribution and its general relation to alfalfa production. Two methods were employed: (*1*) By careful survey it was attempted to discover the peculiar set of conditions under which the disease occurred, and (*2*) by selection of certain fields to produce the disease experimentally under field conditions.

SURVEY STUDIES

The abundance of white-spot in 1926 was first called to the attention of the writer on May 6, two days after the beginning of a heavy rain which fell over the northern part of the state. This was the first rain of consequence which had fallen since April. The various crops, including alfalfa, were suffering heavily as a result of the lack of water. Farmers were beginning to apply the first irrigation water.

On May 7, 8, and 9, fields were studied in the various counties, as follows: Utah, 6; Salt Lake, 10; Cache, 54. A few striking observations will indicate the type of evidence obtained during the survey.

Emil Feulner's Farm, Salt Lake County.—This particular farm was visited on May 7. Irrigation water had flooded part of the west side of the alfalfa patch previous to the visit. The remaining portion had received no water except rain. In the irrigated portion there was white-spot, while none had occurred in the non-irrigated portion.

Joseph Larson's Field, Pleasant Grove, Utah.—In this field, visited on May 7, white-spot areas were conspicuous. Many of the leaves in these areas exhibited incipient lesions, indicating but recent injury. Heavy irrigation had been applied the afternoon and night of May 5, two days prior to the visit.

Fields at Providence, Utah.—Two small alfalfa patches, of approximately 5 acres each, parallel each other. At the time of the visit on May 9 both fields showed serious white-spot injury in an advanced stage. The disease appeared more or less throughout the fields, so that approximately 50 per cent of the plants were affected. In some of the most seriously affected areas 100 per cent of the plants were diseased. The fields had been irrigated on May 4 and 5.

Old Alfalfa Field at Wellsville, Utah.—In a small field of old alfalfa visited on May 13, the disease appeared in an advanced stage on approximately 40 per cent of the plants. It was found that the field had been watered on May 5, one day after the rain.

Fields at Farmville, Utah.—The clearest evidence of the relation of irrigation to white-spot obtained during the survey was found in a study of three small alfalfa fields at Farmville, three and one half miles north of Smithfield, in Cache County. These three fields, of approximately seven acres each, lie adjacent and parallel to each other.

At the time of visit, on May 9, the first field on the north showed heavy white-spot (Fig. 3). White-spot was observed in isolated areas in the center field two days after the first visit but in a less severe form than in the north field (Fig. 4). The south field was entirely free from the disease. Investigation disclosed the fact that the north field had been irrigated heavily on May 4, the day of heavy rainfall; the center field three days later, May 7, two days prior to the first visit; but that the south field had received no irrigation. The south field remained entirely free from white-spot until irrigated on May 27. The following day, May 28, early stages of white-spot lesions were found in abundance, and within 48 hours they had reached their full expression. Other fields with white-spot were visited which showed quite as intimate a relation to irrigation.

During the survey studies careful observations were made as to the state of health of the plants most severely affected. In general, the disease most commonly occurred on plants which were in a vigorous and succulent state

of growth. Plants retarded in growth on account of poor soil or drought occasioned by exposed, gravelly spots in the fields seldom were found affected. This relation was especially clear in the Williamson field (Fig. 3).

No positive correlation was noted between soil type and occurrence of white-spot. The severest form of the disease, however, was found at Farmville, Cache County, on soils derived almost entirely from a dolomitic formation and with a high magnesium content. Again, the disease appeared slightly more abundant in other parts of the state on soils high in calcium. In general, the disease was more abundant and more severe in Cache Valley than in any other part of the state visited.

EXPERIMENTAL STUDIES, 1926

The relation of irrigation to white-spot production was so evident that it appeared entirely possible to produce the disease by the application of irrigation water on fields not previously watered. This was accomplished in abundance during the seasons of 1926 and 1927.

Four fields in Farmville, Utah, were selected for observations. These fields were all examined for the presence or absence of white-spot just prior to the first irrigation. They were then irrigated and subsequently watched to determine the effect of the water on the development of the disease.

On May 14, field No. 1 (8 acres) was carefully examined and found to be entirely free from white-spot. The field was then irrigated during that afternoon, and during the afternoon and night of May 15. The examination was not made until May 20, at which time the writer was confronted with the most severe expression of white-spot he had ever observed. Between 70 and 80 per cent of the alfalfa plants were affected and to a degree varying from but few lesions on the leaves to complete killing of every leaf on all stems of some plants.

In addition, similar lesions were found abundantly on sweet clover (*Melilotus alba*) and occasionally on black medic (*Medicago lupulina*). Upwards of 80 per cent of the sweet clover plants in the areas in which the alfalfa was most severely affected showed lesions. These lesions were comparable to those on the alfalfa in time of occurrence, distribution on the leaf, and in the distribution of the lesioned leaves on the stem. The final color of the lesion on sweet clover was dark brown instead of white or light yellow, as in the case of alfalfa (Fig. 2). The final color expression on the black medic more closely approximated that of the alfalfa lesion.

The second field (No. 2), on which water had run during the night of May 20, was examined in the forenoon of May 21. On this date the very earliest stages of lesion development were found, indicating that the disease developed rather suddenly after irrigation. Full expression of these lesions

resulted in approximately two and one half days from the time of the first observations.

Field No. 3 was examined carefully the evening of May 26, with the result that only one plant within a small area previously flooded was found showing white-spot lesions. Water was allowed to run on part of the field during the next afternoon and evening, May 27. The remaining portion of the field was watered May 28. Examination on the morning of May 29 disclosed a general distribution of white-spot in the early stages of development. Between 25 and 30 per cent of the plants in the field were affected. But a single small area adjacent to a small swamp on which the plants had a constant water supply remained free from the disease. By May 31, the disease had reached its full expression.

Field No. 4, the south field of the three observed at Farmville, was examined on May 27 and found free from white-spot. Water was applied during the night. The early stages of the disease were evident by 9 o'clock the following morning; the disease reached full expression in approximately two days.

FIG. 5.—White-spot affected area in Williamson's field photographed May 14, 1927, at 11:30 A. M., two and one-half hours after the appearance of the first lesion.

EXPERIMENTAL STUDIES IN 1927

In 1927 the studies were confined to five fields in the Farmville district.

On May 10 the writer made his first trip to the Farmville district. At 5:30 P. M., white-spot, in its early stages, was found in a 13-acre field be-

longing to Mr. Hillyard. Water had been turned on this field at 9 A. M., May 9, and had run during the day and part of the night. The first symptom of the disease to be noticed was dropping of the leaves at 3 o'clock the next afternoon (May 10). The field was very severely affected, and the disease caused a loss of approximately one third of the crop. Mr. Hillyard also informed the writer that owing to the severity of white-spot on a 40-acre field in this same district he had been forced to abandon the practice of irrigating the first crop.

During the night of May 10, waste water from the Hillyard farm flooded strips of alfalfa through the north end of the 8-acre field which had been used for experiments in 1926 (field No. 1). Severe white-spot resulted and was confined entirely to the watered area. By request, water was allowed to run during the night of May 11 on additional ground in this same field not previously irrigated. Observations were commenced early the next morning in cooperation with Dr. F. B. Wann and Mr. H. L. Blood of the Department of Botany. At 11:20 A. M., first indications of the disease were noted in the form of opaque pea-green spots in the mesophyll of a few leaves of a single plant. Within 30 minutes similar pea-green opaque lesions appeared over the entire irrigated area. By 2:45 the same afternoon, the disease in this affected portion of the field was distinct enough to be photographed. Bleaching of the lesions was so rapid that by evening of the same day the whitened lesions were clearly noticeable from some little distance. Full expression of the lesions was reached in approximately two

FIG. 6.—Affected spot shown in figure 5, 48 hours after the appearance of the first lesion.

FIG. 7.—Typical white-spot on second-crop alfalfa produced by irrigation, July 4–5, 1927.

and one half days. The remaining portion of this crop was also damaged severely by white-spot from a later irrigation, as in 1926.

At 6 A. M. on May 13, water was started on one division of the field owned by Mr. Williamson.[3] At noon, water was put on a second part of the field, and a third part was irrigated beginning at 5 P. M. The water was allowed to run on all parts during the night and was turned off at 6 A. M., May 14. All three divisions were carefully watched from the time water was turned on the first portion of the field until dark. Obeservations were commenced at daylight on May 14. By 8:30 A. M., the first sign of injury was detected on a single plant in the area watered from 12 (noon) on. Almost simultaneously the lesions were observed on plants in the other two divisions. By 11:30 A. M., the field was photographed, showing distinctly the area affected (Fig. 5). A second photograph was taken on May 16, 48 hours later. (Fig. 6.) Plants watered from 6 P. M. and during the night showed damage as early and of equal severity as those watered for a longer period (Fig. 4).

An adjacent field on the south, rented by Mr. Williamson, the center field of the three studied in 1926, was watered during the night of May 14. Lesions appeared about 9 o'clock the next morning.

At 10 o'clock on the morning of May 15, water was started on one half of the 7-acre field to the south and allowed to run for 10 hours. The water was then turned off and started on the second half, where it was likewise allowed to run for 10 hours (until 6 A. M., May 16). Three hours after the water was turned off lesions were noted on plants in the part irrigated

[3] This was the north field of the three adjacent fields on which observations were made in 1926 and which are recorded in the foregoing discussion.

during the night. In fact, many plants in a number of areas were killed. No white-spot developed on plants watered during the daytime or from 10 in the morning to 8 in the evening, May 15.

Several additional fields were similarly observed after they had been irrigated, with results similar to those detailed. In all cases, lesions were found to develop early in the morning following the irrigation. In no case were lesions found to result during the latter part of the day following daytime watering.

The question naturally arises as to relative sensitiveness of the various crops of alfalfa to possible damage by irrigation. During 1926 the writer noticed slight injury to plants in both second and third crops in the various northern counties surveyed. O'Gara (4) likewise noted the disease on all three crops. In an attempt to study this problem further, the second crop on the 8-acre field (field No. 1, 1926) was irrigated as follows: On Saturday morning July 4, water was turned on the east part of the south half. This ran all day; at night the water was changed to the west division of the south half where it was allowed to run all night. The disease occurred in a very severe form on the part watered during the night. None occurred on that part watered during the day. The north half of the field was watered Monday afternoon and part of the following night, July 4. Lesions appeared abundantly at 8:45 A. M., July 5. The damage on this division of the field was very severe, probably the most severe which had been produced throughout the experiments (Fig. 7). No further tests were made on the second and none on the third crop.

LESION TYPES AND SEQUENCE OF LESION DEVELOPMENT

Descriptions and photographs presented by Clinton (1) and Crabill (2) indicate a peculiar and uniform type of spotting as characteristic of the white-spot disease in New York and Virginia. This same "spot" type of lesion (Fig. 8), likewise characterizes generally the white-spot in Utah, although under certain conditions a very definite marginal injury is found associated with it (Fig. 9). In the first crop, marginal injury may occur on scattered plants in fields where spotting predominates or it may equal in prominence the spot type in the same fields. In 1926, marginal lesions occurred almost exclusively in the second and third crops of alfalfa. In the milder form of the disease marginal injuries may involve but a few cells of the distal portion of the leaf, or under more severe conditions it may involve the entire margin of the leaflet. As with the spot type, in its severest form practically the entire leaflet area becomes affected, leaving but a narrow strip of green along the mid-ribs and a few lateral veins (Figs. 8 and 9). In all cases, as with the spot type of injury, the transition between the diseased area and the healthy portion is very abrupt.

To the writer's knowledge, no worker has described the various stages of lesion development. This phase was carefully observed during the experimental studies. The very earliest expression of the disease appeared in the form of opaque areas in the leaf mesophyll which are especially prominent when the leaf is held between the eye and the source of light. At this early stage no color change is evident. This opaqueness probably results from the substitution of air for water in the injured tissue, the healthy tissue remaining turgid and translucent in appearance. With the substitution of air in the tissues, the lesions sink slightly and rapidly change from deep chlorophyll green to light cress through chrystolite green (6) to a yellowish gray green, and finally with bleaching to white or light yellow or tan. These earlier color changes in the lesions are apparent only on the upper side of the leaf where evaporation is probably most rapid. Later the tissues of the lower side of the leaf go through similar color changes. Finally, within 48–60 hours in dry weather, the affected tissue becomes completely bleached (Fig. 10) and presents a lesion with uniformly smooth and clear surface on both sides of the leaf. On the older leaves, at this time, the affected tissue appears to be dead. In the very young leaflets

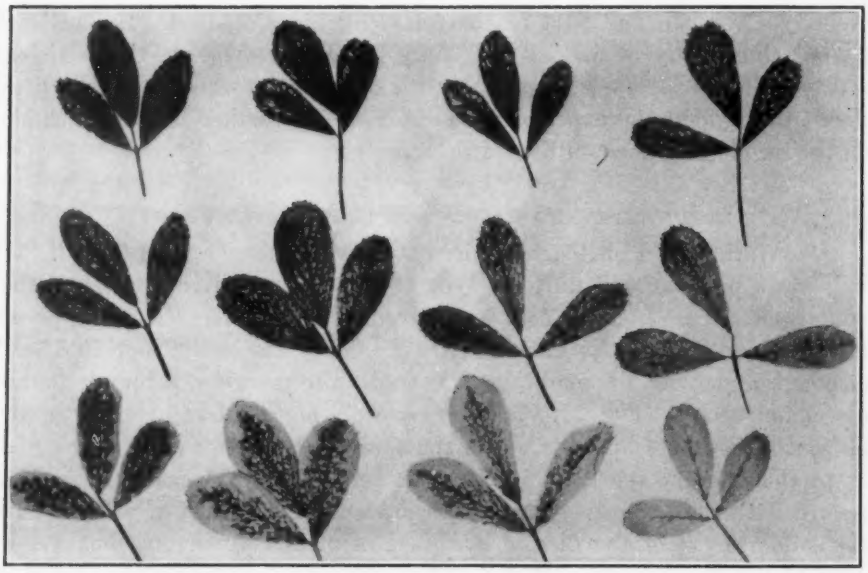

FIG. 8.—Typical white-spot. Beginning with upper left-hand leaf, leaves show gradations from slight injury with few spots to complete whitening of leaf. Veins remain green for some time after bleaching of other parts. This type of lesioning is more common than the marginal type, especially on the first crop, and agrees with white-spot as described by other workers.

at the growing apex, the affected tissue apparently is not killed, and the lesions seldom reach the completely bleached stage. The cells in such affected areas continue to live and in some instances apparently recover their normal green color. Recovery, however, has not been clearly demonstrated. Leaves developed subsequent to the application of irrigation water and the production of the disease are always free from white-spot and are apparently normal.

When the disease develops during rainy weather or during rains subsequent to lesion development, the dead tissue may lose its white appearance and become deep yellow or even dark brown in color. The characteristic color of the lesion is reflected in the appearance of the white-spot area in the field. Fruiting of saprophytes on the dead tissues during rainy periods may add to the color changes.

Injury to the plant may be but slight or the death of the entire plant may result. When injuries are medium to severe, but not resulting in stem destruction, leaf fall may become a serious factor in decreasing the yield and the feeding value of the crop. In a series of counts on a large number

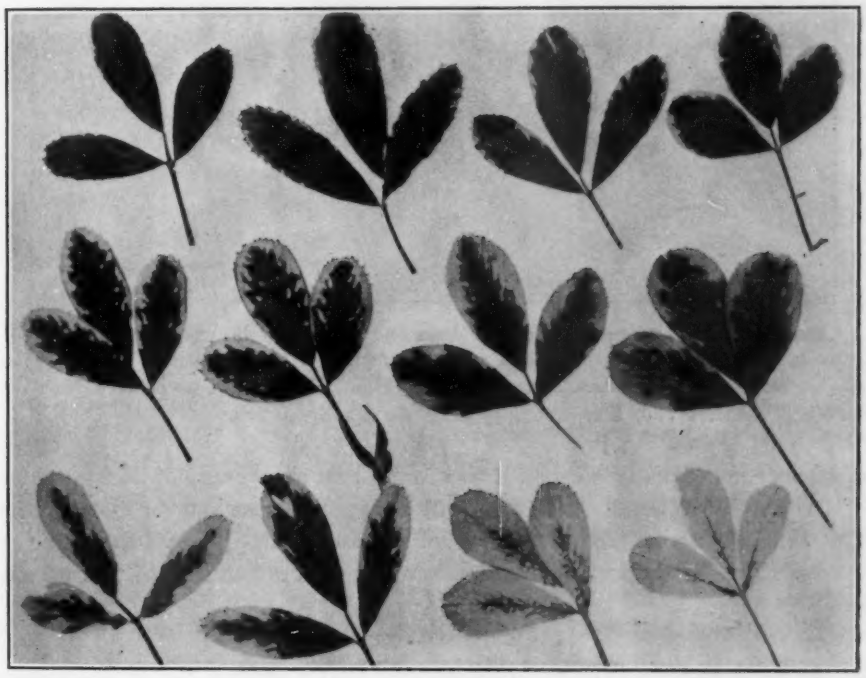

FIG. 9.—Alfalfa leaves showing various degrees of marginal injury produced by irrigation water. The leaves were taken from different plants on the Carbridge 8-acre farm 5 days after irrigation.

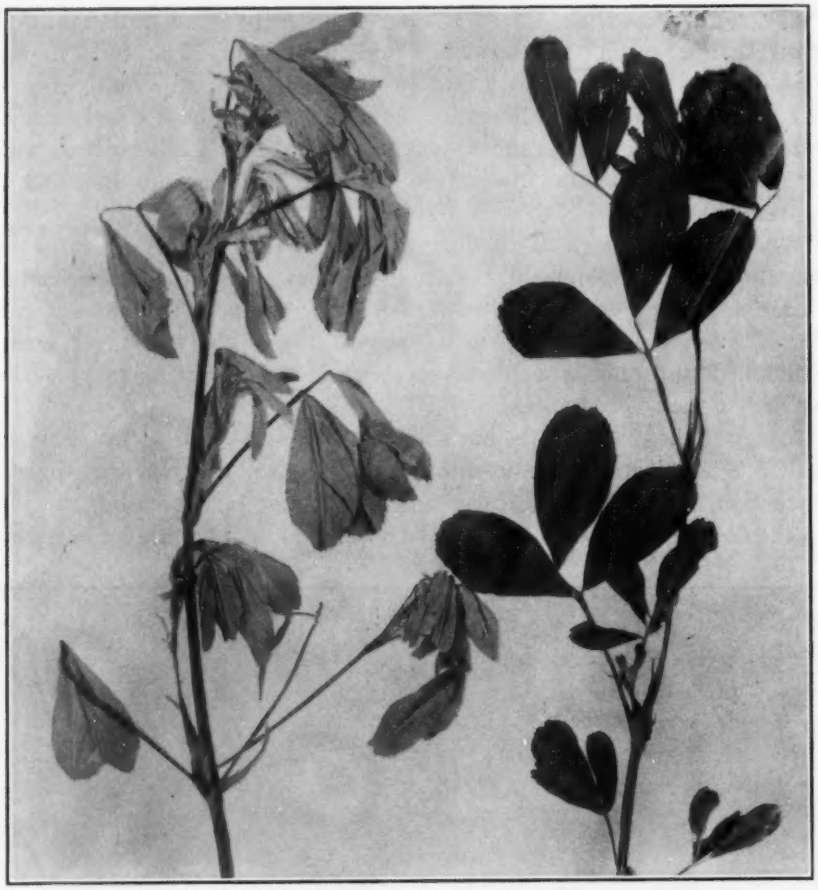

FIG. 10.—Alfalfa stem at left with dead and completely bleached leaves resulting from irrigation. The upper petioles and the younger apical portion of the stem has also succumbed to the disease. Normal stem at right. Both stems were taken from the Carbridge field May 20, 1926, five days after the first irrigation.

of stems about equally affected it was found that an average of 26.5 per cent of the leaves dropped. This leaf fall is especially serious in view of the fact that approximately two thirds of the food value of alfalfa is in the leaves. Farmers report that animals avoid hay affected with white-spot.

DISCUSSION OF RESULTS

As early as 1918 O'Gara (4) suggested that a sudden rise in the water-table in irrigated districts may produce white-spot in alfalfa, and further observed that "irrigated fields showed white-spot trouble, while non-irri-

gated fields showed none." O'Gara describes no specific observations. The experimental results detailed in the foregoing pages, however, leave no doubt as to the accuracy of O'Gara's observations.

Survey data of 1926 suggest that white-spot may result from heavy rainfall, especially when plants are in a state of drought. This conclusion is strongly supported by observation in Michigan. Nelson, of the Michigan Experiment Station, who sent specimens of the disease to the writer, states that the disease was found coincident with a heavy rainfall.

In general, the data indicate that an unbalanced water relation in the soil and subsequently in the plant is an important cause of white-spot and that this condition induced by irrigation is the chief cause of white-spot in the semi-arid West.

From the literature it would appear that factors other than a disturbed water relation are involved in the production of white-spot. Crabill (2) correlates the disease with crown injury and claims to have produced it by artificially mutilating the crown. O'Gara (4) reports the production of white-spot in his experimental plots by watering plants with various solutions. O'Gara does not report details of the experiments: neither does he claim to have checked his experiments by watering plants without substances in solution. In addition, he also correlates the disease with excess alkali. In areas adjacent to smelters white-spot is not infrequently but probably erroneously attributed to injury from sulphur dioxide or other fumes emitted by smelting plants.

It is conceivable that a number of factors may be responsible for white-spot and that mesophyll lesions are a generalized symptom related in a variety of ways to these various etiological factors.

It is significant that injury from irrigation characterized by alfalfa white-spot is not peculiar to this particular plant. It will be recalled that lesions corresponding in nature, time, and sequence of development appeared on both sweet clover and black medic under conditions identical with white-spot lesions of alfalfa (Fig. 2). The lesions on these plants were quite as definitely related to irrigation as were the typical lesions of white-spot on alfalfa plants. It is not unlikely that in white-spot we have a generalized type of injury resulting from seriously unbalanced water relations, common possibly to a large number of plants. With respect to etiology, it is not improbable that such diseases as late blight of sugar-beet, tip burn of lettuce (8), and black heart of celery will fall into this class.

Just how excess water in the soil resulting from heavy rain or the application of irrigation water produces the disease spoken of as white-spot is not clear. Some have suggested that possible absorption of deleterious salts, or salts in excess quantities, which, when left behind in the leaf after

evaporation, kill the mesophyll tissue. A second explanation lies in the possibility that rapid absorption of water may actually burst the mesophyll cells and chloroplasts. A third theory is suggested in the degree to which the sudden filling of the tissues with water resulting from rapid absorption and from the depression of evaporation with increased humidity might decrease the oxygen supply in the plant and thereby interfere with respiration. The by-products of incomplete respiration or inhibited respiration may thus poison and kill the living cells. While it is quite possible that none of these theories offers the correct explanation, the sudden and uniform appearance of the disease, together with its possible relation to the depression of transpiration during the night, seems to support the latter view. On the other hand, the appearance of the disease year after year in the same field suggests the salt-absorption theory. The answer to the question provides an interesting problem for research on which the Department of Plant Pathology of the Utah Agricultural Experiment Station is placing some effort.

SUGGESTIONS FOR CONTROL

To date, experimental studies have not been made with respect to control of white-spot. A few suggestions may be derived, however, from the nature of the disease and the conditions under which it occurs. As a more severe expression of the disease results from excess irrigation following drought, it would seem good practice with the first crop, in areas of annual occurrence of the disease, to water a little earlier in the season, using lighter applications a little more frequently than is commonly the practice. A uniform condition of moisture in the soil appears definitely to lessen the danger from the disease. It is quite possible that in the areas most severely affected by irrigation, the avoidance of night irrigation may aid in preventing the disease. To what extent this latter suggestion will be helpful is not determined. When the disease is so severe as seriously to retard growth of the crop in the affected area, early cutting may be justified in order that the succeeding crop may be given an early start.

SUMMARY

White-spot varies greatly in economic importance throughout the United States where it is known to occur. In certain areas it is of no significance, while in local areas in the West where irrigation is necessary the disease may become a serious factor in alfalfa production.

Affected plants are retarded in growth, have less effective leaf surface, and under severe conditions are killed. Succulent, rapidly growing plants are most severely damaged. Seldom is the stunted and drought-conditioned plant affected. Lesions are of two types: (*1*) spotting due to localized

killing of the mesophyll tissues between the veins, and (*2*) marginal tissue injury which may affect but a few apical cells of the leaf or may involve the entire leaf margin. Both types may be so severe as to involve the entire leaf surface.

Survey data procured during 1926 showed clearly that white-spot of alfalfa is greatly influenced by rainfall and irrigation. These observations were definitely confirmed by experiments during 1926 and 1927. During these years the disease was produced over a considerable acreage in a number of different fields at different periods and on both the first and second crops. In the various experiments lesions of white-spot were found to occur simultaneously on plants in affected areas in the irrigation fields, usually early in the forenoon after the water had run during the previous night.

Earlier, more frequent, and lighter irrigations are suggested as possible aids in preventing the disease in the first crop.

UTAH AGRICULTURAL EXPERIMENT STATION,
LOGAN, UTAH.

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PHYSIOLOGIC SPECIALIZATION IN FUSARIUM SPP. CAUSING HEADBLIGHT OF SMALL GRAINS

CHIH TU[1]

INTRODUCTION

The Fusarium headblight (scab) of wheat and other cereals, together with seedling blight caused by similar fungi, is an important factor in cereal growing in many countries of the world. In the United States wheat scab causes heavy damage in the soft winter wheat area and in the spring wheat area. Although the disease has been investigated extensively, no really satisfactory control measures have been devised. The development of resistant varieties apparently is the only adequate method of control. Very little is known, however, about the species of *Fusarium* which cause the disease and about the possible physiologic specialization within the different species. The present investigation was undertaken primarily to determine the relative virulence of the different species of *Fusarium* that cause headblight and to ascertain whether there are physiologic forms differing in pathogenicity and in their relation to environmental conditions. This knowledge is basic to a sound program of breeding disease-resistant varieties.

METHODS AND MATERIALS

Isolations were made primarily from blighted kernels of wheat and oats. The kernels were soaked in 70 per cent alcohol for two or three minutes and then burned over a free flame until slightly charred. The surface-sterilized kernel was then placed in a petri dish containing a suitable medium. Usually a colony of *Fusarium* grew sufficiently large in three or four days to be transferred to test tubes for stock cultures. Stock cultures were stored either in the ice box at 10–12° C. or at room temperature ranging from about 18° to 22° C. All of the experiments were made with monosporous cultures. Sherbakoff's method of single-spore isolation was used exclusively (19, p. 104).

For all inoculation experiments, cultures were grown in Erlenmeyer flasks on a sterile mixture of equal parts of wheat and oat seed. Usually 25 gms. of seed to 50 cc. of water were placed in a 250-cc. flask and auto-

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TABLE 1.—*Sources of the Fusaria of cereals studied*

Fungus	Original host	Place of collection	Date of collection	Previous history
Fusarium graminearum Schwabe form 1	Wheat	Wisconsin	1924	Dr. L. R. Jones' culture, furnished by Dr. Louise Dosdall
do form 2[a]	do	Waseca, Minn.	Aug. 21, 1926	Material furnished by Dr. O. S. Aamodt
do form 3[a]	Oats	Purgatory Springs, Minn.	Aug. 10, 1926	Material furnished by Dr. J. J. Christensen
F. culmorum (W. G. Sm.) Sacc. form 1[b]	Wheat	University Farm, St. Paul, Minn.	July 10, 1926	do
do form 2[b]	do	do	Sept. 16, 1926	do
do form 3[b]	do	Purgatory Springs, Minn.	Sept. 20, 1926	do
F. avenaceum (Fr.) Sacc. form 1	do	Ithaca, N. Y.	Aug. 17, 1926	Material furnished by Dr. E. C. Stakman
do form 2	do	do	do	Culture furnished by Dr. C. D. Sherbakoff
F. solani (Mart. P. Par.) Ap. et. Wr.	do	do	do	do
F. nivale Sor.	Rye	Schlesien, Germany	Summer, 1925	Culture furnished by Dr. H. W. Wollenweber

[a] Cultures identified by Dr. C. D. Sherbakoff.
[b] Cultures identified by Dr. H. W. Wollenweber.

claved for half an hour at 15 lbs. pressure. In making head inoculations, a spore suspension was used, which was made by washing the culture with a definite amount of sterile water and then straining the washing through sterilized cheesecloth.

The seeds of cereals were obtained from Professor A. C. Arny, Division of Agronomy, University of Minnesota. All the seeds used were presoaked in water from 8 to 12 hours and then treated with 0.25 per cent solution of Semesan for 4–5 hours.

The sources of the different Fusaria used in the studies are listed in table 1. Three cultures of *Fusarium graminearum* Schwabe [*Gibberella saubinetii* (Mont.) Sacc.], three of *Fusarium culmorum* (W. G. Sm.) Sacc., and two of *Fusarium avenaceum* (Fr.) Sacc. were found to comprise physiologic forms distinguishable on the basis of their pathogenicity. For convenience these cultures are designated as physiologic forms 1, 2, etc., as in the list. *Fusarium culmorum* form 1 mutated in the course of study and the resulting culture is listed as *F. culmorum* form 1 mutant.

PATHOGENICITY AND PHYSIOLOGIC SPECIALIZATION

The fact that different cereal varieties differ in their degree of susceptibility to Fusarium headblight has long been recognized by investigators of the disease (3, 4, 5, 9, 10, 12, 16, 17, 18, 20). As a result of extensive varietal tests carried on for eight years at University Farm, St. Paul, Minnesota, it was found that the amount of headblight in individual varieties of wheat varied from year to year and in different localities in the same year (9, 10). Christensen and Stakman (9) suggested that "There was also some circumstantial evidence that different parasitic strains may have been present in different years," in order to account for the different results obtained.

In studying the root rot of wheat, Henry (15) found that different strains of *Fusarium moniliforme* Sheldon differed in virulence on seedlings. Recently Greaney and Bailey (14) tested the pathogenicity of 28 cultures of unidentified Fusaria in the greenhouse and found that there were great differences in the degree of virulence on wheat seedlings.

The writer made a comparative study in 1927 of the pathogenicity of six cultures of *Fusarium*, three of *F. culmorum*, and three of *F. graminearum* (*Gibberella saubinetii*). Chiefly the headblight phase of the disease was studied.

In the head-inoculation experiment in the field seven varieties of wheat (Marquis, Minn. 1239; Preston, Minn. 188; Kota, C. I. 5878; Ceres, N. D. 1658; Glyndon Fife, Minn. 163; Pentad, C. I. 3322; Mindum, Minn. 470) and one variety of barley (Minsturdi, Minn. 439) were used as differential

TABLE 2.—*The percentage of headblight, caused by three physiologic forms of Fusarium culmorum and three of Fusarium graminearum in one variety of barley and seven varieties of wheat grown at University Farm, St. Paul, Minnesota, in 1927*

Inoculum	Variety and percentage of head infection						
	Minsturdi barley	Marquis	Preston	Kota	Ceres	Glyndon Fife	Pentad	Mindum
Fusarium culmorum form 1	94.5	5.5	0.0	0.5	0.1	0.0	0.0	1.0
do form 2	61.4	1.1	0.0	0.6	3.9	0.0	3.6	0.0
do form 3	79.5	14.3	17.5	16.2	37.6	4.8	27.3	3.0
F. graminearum form 1	78.6	22.1	23.4	1.6	44.3	6.4	34.9	4.9
do form 2	32.3	0.2	13.2	0.3	0.5	0.0	7.0	1.9
do form 3	18.7	0.0	0.2	0.3	0.2	0.0	1.3	0.5
Check	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

hosts. The eight differential hosts were grown side by side in three 7-foot rows and replicated twice in each plot. The seven plots were systematically distributed in the field in order to reduce the danger of contamination. Each plot was sprayed with a spore suspension of a different culture. The first spraying was done at flowering time and four subsequent sprayings were made at intervals of three or four days, while the check plot was sprayed with sterile water. Spraying was usually done in the evening, when the temperature was relatively low. The results are summarized in table 2.

On the basis of the degree of infection produced by the different cultures, three physiologic forms of *F. culmorum* and three of *F. graminearum* were distinguished. It is interesting to note that headblight in Minsturdi barley ranged from 18.7 per cent when *F. graminearum* form 3 was used as inoculum, to 94.5 per cent when *F. culmorum* form 1 was used. *F. graminearum* form 1 and *F. culmorum* form 3, producing 78.6 and 79.5 per cent headblight respectively, ranked next in virulence on barley. Form 2 of *F. culmorum* and form 2 of *F. graminearum* both were still less virulent on barley. The former produced 61.4 per cent infection, while the latter produced only 32.3 per cent. On wheat the percentage of infection was never so high as on barley. On all varieties of wheat, except Kota, *F. graminearum* form 1 produced consistently higher percentages of hedblight than any of the other cultures. On Kota wheat *F. culmorum* form 3 produced 16.2 per cent headblight as compared with 1.6 per cent produced by *F. graminearum* form 1. *F. culmorum* form 3 was the next most virulent form on wheat.

The rather low percentages of headblight on the wheat varieties inoculated may have been due to the hot, dry weather which followed the flowering period.

In 1928 a similar but more extensive head-inoculation experiment was made. In addition to the cultures used in the previous year, five more were tested, including *F. nivale* (*Calonectria graminicola*), *F. solani,* two physiologic forms of *F. avenaceum,* and *F. culmorum* form 1 mutant. As differential hosts, two varieties of barley (Minsturdi; and Glabron, Minn. 445), seven varieties of wheat (Marquis; Preston; Kota; Ceres; Haynes Bluestem, Minn. 169; Mindum; and Akrona, C. I. 6881) and two varieties of oats (Victory, Minn. 564; and Anthony, Minn. 686) were used. The differential hosts were grown side by side in rod rows in each plot, and the plots were systematically replicated twice. The plants were inoculated in the same manner as those grown the previous year. The results are summarized in table 3.

TABLE 3.—*The percentage of headblight caused by 11 cultures of Fusarium in 2 varieties of barley, 7 varieties of wheat, and 2 varieties of oats grown at University Farm, St. Paul, Minnesota, in 1928*

Inoculum	Variety and Percentage of Infection										
	Barley		Wheat							Oats	
	Minsturdi	Glabron	Marquis	Preston	Kota	Ceres	Haynes Bluestem	Mindum	Akrona	Victory	Anthony
Fusarium culmorum form 1	87.4	89.3	45.5	6.5	42.6	22.4	21.1	48.0	62.3	64.1	62.1
do form 1 mutant	28.8	56.1	10.6	14.2	4.1	7.6	4.5	18.5	8.0	46.2	43.0
do form 2	42.4	63.3	29.5	26.2	14.0	28.0	16.6	15.1	27.5	40.1	39.2
do form 3	85.5	74.8	26.2	41.5	58.1	47.6	47.5	63.5	76.5	70.1	68.2
F. avenaceum form 1	39.6	47.8	17.0	3.1	8.3	7.6	21.0	27.2	26.5	37.0	51.5
do form 2	49.0	60.0	31.0	18.2	25.4	31.0	32.5	15.4	16.1	47.2	53.3
F. solani	39.5	61.6	19.5	10.3	13.5	14.1	17.6	12.2	20.1	27.3	46.1
F. graminearum form 1	84.4	88.8	82.1	23.1	46.0	51.6	24.5	57.3	57.5	49.1	58.2
do form 2	40.8	50.7	46.1	6.5	30.5	21.3	15.6	28.5	54.0	32.4	56.2
do form 3	32.6	44.1	15.6	13.3	9.1	9.6	8.5	16.0	27.5	78.0	80.2
F. nivale	48.2	62.4	25.1	18.1	29.1	15.1	24.1	9.0	25.3	42.1	54.2
Check	4.1	5.6	3.2	2.1	3.4	3.0	1.5	2.9	2.0	3.2	4.1

The experiments of 1928 confirmed the results obtained in 1927. From table 3 it is evident that the relative virulence of the physiologic forms both of *F. culmorum* and *F. graminearum* on barley and wheat was practically the same as that in 1927, except that the percentages of infection on wheat were generally higher in 1928 than in 1927. It seems apparent that the relatively successful infection of wheat may have been due to the cloudy and rainy weather following the flowering period in 1928. The ability of the various Fusaria to infect oats varied. *Fusarium graminearum* form 3 was the most virulent form on oats, producing 78.0 per cent on Victory and 80.0 per cent on Anthony, while it was the least virulent on barley and weak on most of the wheats. *Fusarium culmorum* forms 3 and 1 produced nearly as much infection, while *F. graminearum* form 2 was the least virulent on Victory and *F. culmorum* form 2 least virulent on Anthony.

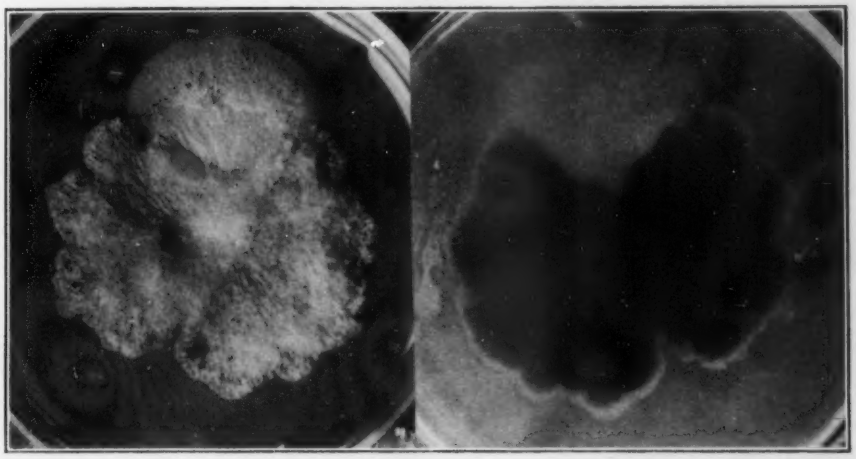

FIG. 1. The mutant of *Fusarium culmorum* form 1. Left: top view; right: bottom view.

The two physiologic forms of *F. avenaceum* differed in their pathogenicity, although they were weak in comparison with other virulent cultures. On barley and oats, and on wheat except the durums, form 2 was consistently more virulent than form 1: the former produced 27.2 per cent infection on Mindum and 26.5 per cent on Akrona, while the latter produced only 15.4 and 16.4 per cent respectively.

Fusarium solani and *F. nivale* were comparatively intermediate in their virulence.

MUTATION

Mutation is a fairly common phenomenon in most of the fungi which have been thoroughly studied in this respect. Appel and Wollenweber (12), in their monograph on the genus *Fusarium*, described certain results which indicated that mutation was taking place, although the authors apparently did not recognize it as such. Later, Sherbakoff (19) probably obtained doubtful cases of mutation in his studies on Fusaria of potatoes. Recently Brown (6, 7) found that "saltation" was a fairly frequent phenomenon in various strains of *Fusarium fructigenum* Fr. on concentrated Richards' solution agar. The present author found, in the course of

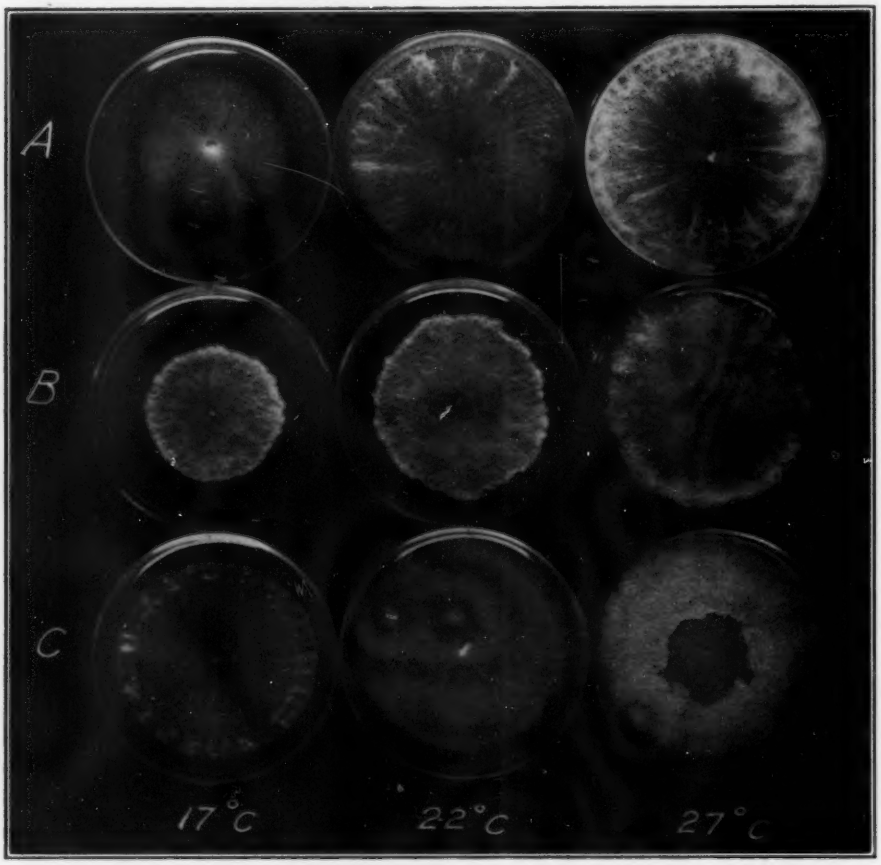

FIG. 2. Effect of temperature on the growth of two physiologic forms of *Fusarium culmorum* and the mutant on Difco potato dextrose agar: (A) *Fusarium culmorum* form 3, (B) *F. culmorum* form 1, (C) mutant of *F. culmorum* form 1. Picture taken 5 days after inoculation.

temperature studies, that *F. culmorum* form 1 mutated at 27° C. (Fig. 1). The mutant differed from the parent in type and rate of growth of the mycelium and in color production at various constant temperatures (Fig. 2). The mutant also produced comparatively fewer spores than the parent, and was much less virulent than the parent in causing headblight of cereals. (See table 3.) However, the factors governing the frequency of mutation on culture media are not very well known at present. Further work along this line is being carried on.

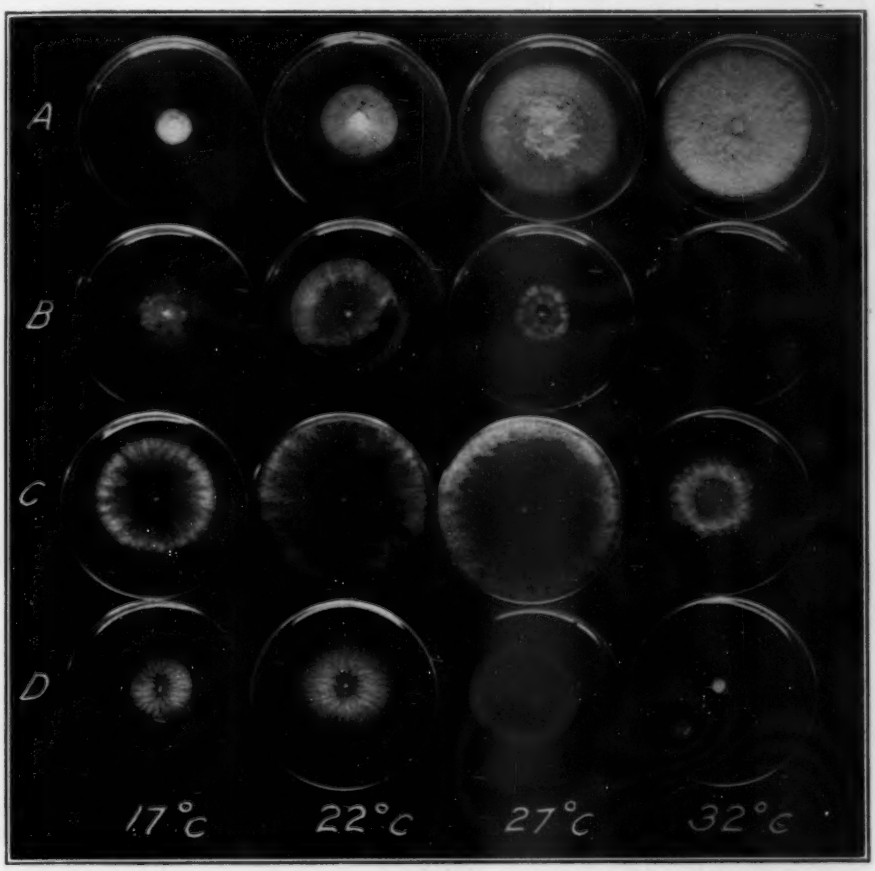

FIG. 3. Effect of temperature on the growth of four species of *Fusarium* on Difco potato dextrose agar: (A) *Fusarium solani*, (B) *Fusarium nivale*, (C) *Fusarium graminearum*, and (D) *Fusarium avenaceum*. Photographed five days after inoculation.

EFFECT OF TEMPERATURE ON GROWTH

The effect of temperature on the mycelial growth of *F. graminearum* was studied by various workers (11, 16). It was found that the organism has the cardinal temperatures of 3°, 25–27°, and 33° C. However, different species of *Fusarium* of cereals may have different temperature requirements, as suspected by Atanasoff (5). Thus a comparative study of the various Fusaria was undertaken by the author. In making the experiment, petri dishes of uniform size were poured with uniform amounts of Difco potato dextrose agar, usually about 18 cc. Bits of mycelium of the cultures to be tested were transferred to the center of the plate. The plates were inoculated in duplicate and were incubated at room temperature for 12 hours before being subjected to the various temperatures. For five days the diameter of each colony was measured daily. The averages of the three experiments for the fifth day are summarized in table 4.

TABLE 4.—*Effect of temperature on the growth of various Fusaria*

Fungus	Temperature in degrees C.					
	7	12	17	22	27	32
	Growth in millimeters 5 days after inoculation					
Fusarium culmorum form 1	14.5	35.0	45.1	57.3	64.5	34.0
do form 1 mutant	11.0	34.7	47.2	84.5	86.5	32.5
do form 2	15.0	42.1	45.7	59.4	68.3	32.5
do form 3	14.2	40.3	59.5	84.1	89.9	22.3
F. avenaceum form 1	13.2	35.7	45.6	60.5	42.3	14.1
do form 2	12.0	23.5	28.7	40.8	49.9	19.5
F. solani	8.1	10.6	18.5	34.2	64.7	71.8
F. graminearum form 1	15.2	37.0	55.3	83.1	89.2	41.5
do form 2	15.4	25.6	42.3	89.1	98.9	35.7
do form 3	12.5	35.2	40.5	52.8	67.5	23.1
F. nivale	14.3	31.4	34.7	48.5	25.3	.....

It was found that some of the Fusaria had different temperature requirements, although most of them were similar to *F. graminearum* in this respect. Two interesting cases were *F. solani* and *F. nivale*. The optimum temperature of the former was about 32° C., while the latter grew best at

about 22° C. and did not grow at all at 32° C. (See figure 3.) It was also interesting to note that the two forms of *F. avenaceum* differed in their temperature requirements: form 1 grew best at 22° C., while form 2 grew best at 27° C., as most of the Fusaria did.

DISCUSSION AND CONCLUSIONS

As a result of the preliminary study of 10 cultures of Fusaria of cereals, experimental evidence proves that there are at least 3 physiologic forms of *Fusarium culmorum*, 3 of *Fusarium graminearum*, and 2 of *Fusarium avenaceum*, which are differentiated by their pathogenicity on different varieties of cereals. There also were differences in pathogenicity among the different species of *Fusarium* studied. Further, it was shown that different Fusaria may have different optimum temperatures for growth. From the results thus far obtained it is quite possible that different physiologic forms, or even different species of *Fusarium*, might predominate in different years on account of variation in weather conditions. If such were the case, the confusing results in breeding work encountered at present might be explained to some extent. Of course, the effect of environment on the development of the host plant and its resistance to Fusarium attack probably is important also.

The occurrence of mutation in *Fusarium culmorum* form 1 may complicate the problem further, for it may alter the pathogenicity of the organism considerably.

SUMMARY

1. A preliminary, comparative study of 10 different Fusaria of cereals was made.

2. Experimental evidence proves that there are three physiologic forms of *Fusarium graminearum* (*Gibberella saubinetii*), 3 of *Fusarium culmorum*, and 2 of *F. avenaceum* which can be differentiated by their parasitism on varieties of cereals. The two physiologic forms of *F. avenaceum* also have different temperature requirements.

3. There also were differences in pathogenicity in the five species of *Fusarium* studied.

4. Mutation occurs in *Fusarium culmorum* form 1 on culture media.

5. The optimum temperature for growth of *Fusarium avenaceum* form 2, three physiologic forms of *Fusarium graminearum* (*Gibberella saubinetii*), and three of *Fusarium culmorum* and its mutant on Difco potato dextrose agar is about 27° C. *Fusarium avenaceum* form 1 and *Fusarium nivale* (*Calonectria graminicola*) have an optimum of about 22° C., and *Fusarium solani* an optimum of about 32° C.

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THE INFLUENCE OF TEMPERATURE ON THE FREQUENCY OF MUTATION IN HELMINTHOSPORIUM SATIVUM

J. J. CHRISTENSEN[1]

INTRODUCTION

Recently the effect of environmental factors on mutation has received considerable attention. There is good evidence that the frequency of mutation in fungi can be stimulated in various ways. According to Brierley (1), Arcichovskij, Waterman, and Schiemann claim to have induced mutation in *Aspergillus niger* and *Penicillium glaucum* by the addition to the culture medium of such chemicals as zinc sulphate and potassium bichromate and by the exposure of cultures to high temperatures. Brierley (1) repeated much of their work but was unable to confirm their results. Christensen (3 and 4) has shown that a given form of *Helminthosporium sativum* may mutate more frequently on one nutrient medium than on another. He also found that some of the physiologic forms mutated readily, while others did not. Brown (2) concluded that a medium which combines high nutrient concentration with minimal staling capacity was conducive to mutation in Fusaria. It also has been demonstrated (3 and 4) that the amount of medium affects the frequency of mutation in *H. sativum*, frequency being greatest when small amounts are used.

In preliminary experiments the writer obtained circumstantial evidence that temperature affected the frequency of mutation in *H. sativum*. A detailed study therefore was undertaken to determine definitely the effect of temperature on mutation in this species.

METHODS

All of the parasitic races of *H. sativum* used in these experiments were monosporous cultures; and in some cases the cultures were obtained by a series of successive single-spore isolations. The numbers used to designate the forms correspond to those listed in table 5 of the author's earlier publication (4).

[1] Published with the approval of Director as paper No. 811, of the Journal Series of the Minnesota Agricultural Experiment Station.

The writer wishes to express his appreciation to Dr. E. C. Stakman for help and criticism in preparing this paper.

The culture medium consisted of 18 gms. of Difco potato dextrose agar and 10 gms. of Difco cornmeal agar in 1 liter of distilled water. The medium for each experiment was made up in one lot, tubed and sterilized. All petri dishes were poured at the same time. In each experiment triplicate series of standard, 90-mm. petri dishes, each containing 18 cc. of the medium, were inoculated in the usual manner with the individual forms. After inoculation, the plates were kept at room temperature for one day and then placed in their respective incubators.

Two experiments were made: the second, one year later than the first. These duplicate series were incubated in two different buildings to eliminate in as far as possible the effect of gases, variation in humidity, and other factors which might possibly stimulate mutation.

RESULTS

The first test was made in March, 1927, with six physiologic forms: 1, 9, 10, 12, 22, and 35. Triplicate plate cultures of each form were incubated at each of the following temperatures: 3°, 9°, 15°, 20°, 25°, 27°, 30°, and 35° C. Twelve days after inoculation the number of sectors in each set of three cultures was counted. After the readings were completed

TABLE 1.—*The number of sectors produced by six physiologic forms of Helminthosporium sativum incubated at eight different temperatures, March, 1927*

Temperature, degrees C.	Physiologic forms						Total no. sectors at each temperature
	1	9	10	12	22	35	
	Number of sectors[a]						
3	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0
20	0	0	1	0	0	0	1
25	19	35	16	0	21	13	104
27	8	49	10	2	9	4	82
30	0	0	0	21	0	0	21
35	0	0	0	0	0	0	0
Total no. sectors in each form	27	84	27	23	30	17	208

[a] Total number of sectors produced in three petri dish cultures.

the cultures kept at the lower temperatures were returned to their respective chambers and incubated until their radial growth was approximately the same as the growth of cultures in which the greatest number of mutations occurred. The additional incubation period lasted from one to three weeks, depending on the temperature at which the cultures were kept, but those kept at 3° C. never achieved the radial growth which the other cultures did. Readings were again made, but in no case was there an increase in the number of sectors over that in the original count. The results obtained are presented in table 1 and figure 1. In each case the number represents the total number of sectors, or apparent mutations, which appeared in a set of three plate cultures.

It is obvious from table 1 that temperature has a profound effect on the frequency of mutation. Thus in the 18 colonies kept at 25° C. there de-

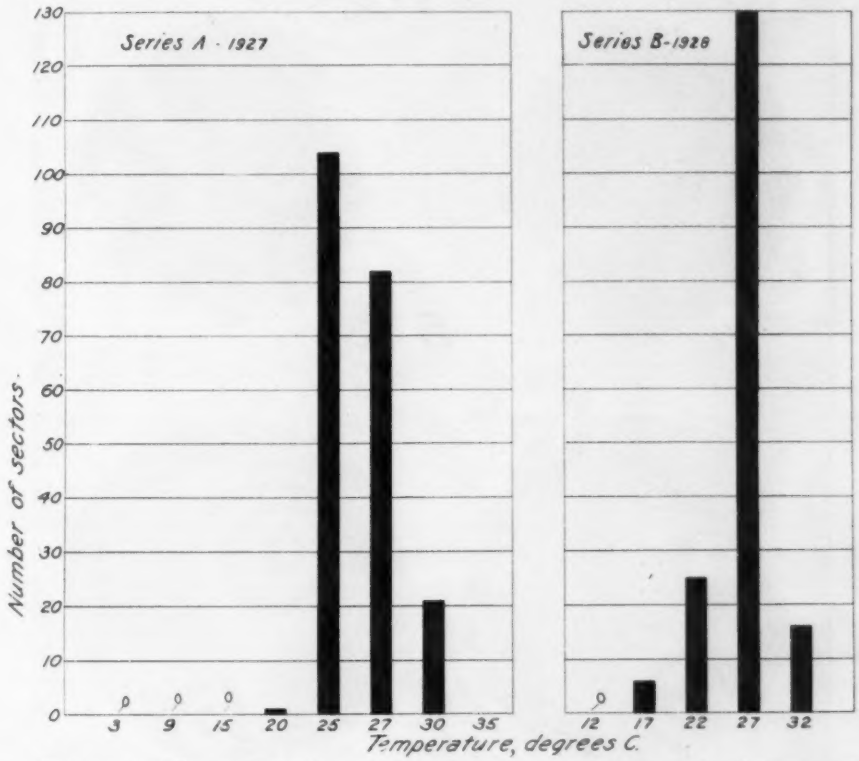

FIG. 1. Graphic representation of the effect of temperature on the frequency of mutation in *Helminthosporium sativum* growing on cornmeal-potato-dextrose agar. Series A and B represent the total number of sectors produced by six and four physiologic forms, respectively.

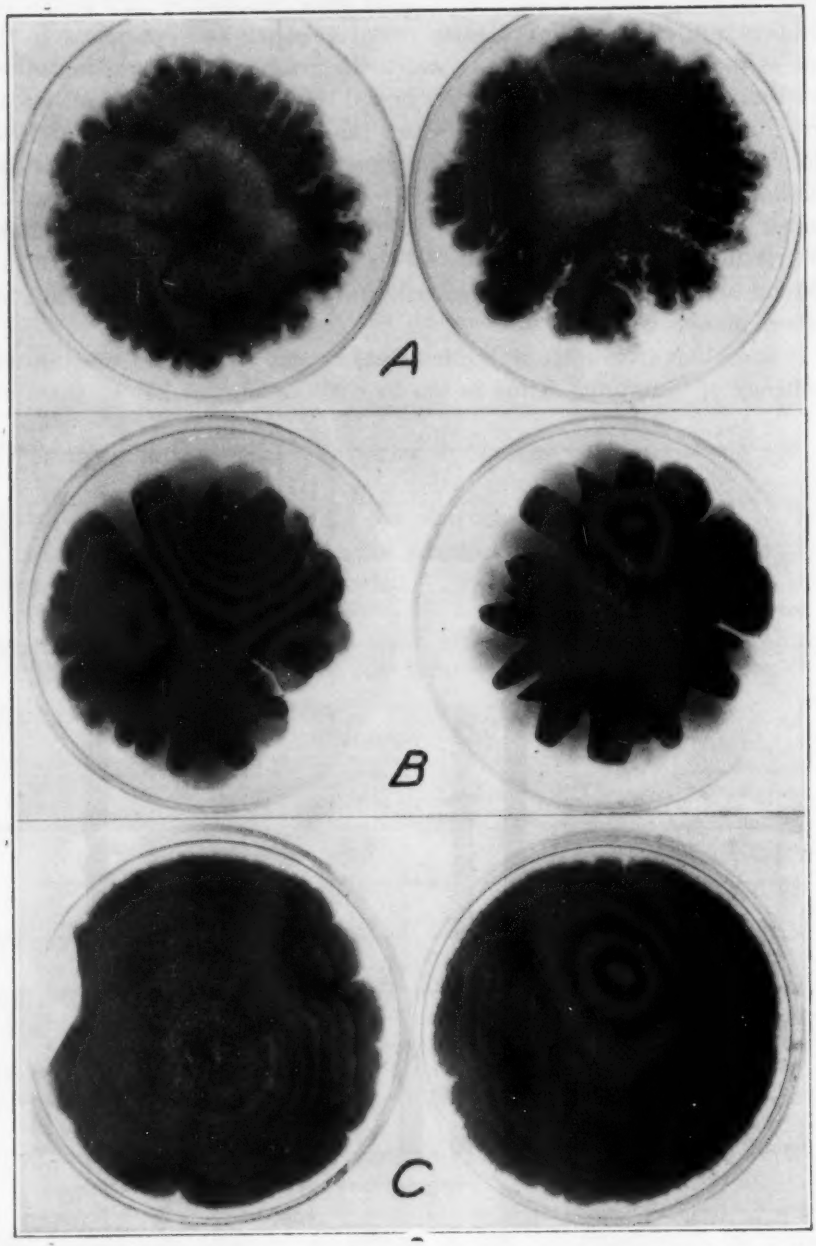

FIG. 2. The effect of temperature on the frequency of mutation in *Helminthosporium sativum*, form 9, growing on cornmeal-potato-dextrose agar, March, 1927. A, B, and C show cultures grown at 15°, 27°, and 30° C., respectively. Note the numerous sectors shown in B.

veloped 104 sectors; in the 18 colonies at 27° C., 82; in those at 30° C., 21; and in those at 20° C. only 1. No sectors were produced at the lower temperatures, 3°, 9°, and 15° C., or at the highest temperature, 35° C. (See figure 2.) At 3° and 9° C. the colonies grew very slowly during the entire experiment, but at 35° C. they grew slowly at first and then stopped growing after a few days.

In April, 1928, a similar test was made, but only four of the six forms previously used were included. Cultures were incubated at the following temperatures: 12°, 17°, 22°, 27°, and 32° C. The data are given in table 2 and graphically presented in figure 1. The results obtained in 1928

TABLE 2.—*The number of sectors produced by four physiologic forms of Helminthosporium sativum incubated at five different temperatures, April, 1928*

Temperature, degrees C.	Physiologic forms				Total no sectors at each temperature
	1	9	10	22	
	Number of sectors				
12	0	0	0	0	0
17	1	0	3	2	6
22	3	2	13	7	25
27	26	43	28	33	130
32	0	12[a]	4	0	16
Total no. sectors in each form	30	57	48	42	177

[a] Nine of these mutants occurred in one colony, the development of which was arrested by some unknown factor.

agreed with those obtained in 1927, although the range of temperatures at which mutation occurred was somewhat wider in 1928 than in 1927. In 1928 sectors appeared in cultures kept at 17° C. and at 32° C.

This experiment again tended to prove that temperature exerts a tremendous influence on frequency of mutation. The optimum temperature for mutation in the 1928 series was 27° C. Unfortunately in 1928 no cultures were incubated at 25° C., which was the optimum temperature for mutation in the 1927 series.

The question naturally arises as to whether these sectors are true mutations or only modifications—temporary phenotypes—induced by environmental factors. The writer (3 and 4) has previously presented evidence that such sectors, at least in many cases, were the result of real genotypic

changes. Since that time more evidence has been obtained in support of this conclusion. A number of mutants of *H. sativum*, together with their respective parents, have been cultured for from one to four years under various conditions. Parents and their mutants have been exposed to conditions as nearly identical as possible. Nearly all of the mutants are to-day as distinct in cultural characteristics as they were on their first transfer (Fig. 3). Two of the mutants now appear to be identical with the parent

FIG. 3. Parents and respective mutants growing in the same plates on 1 per cent glycerin-potato-dextrose agar, three and one half years after isolation of the mutant. A. Bottom: parent, form 22. Top: mutant, M–40. B. Bottom: parent, form 1. Top: mutant, M–1.

strain, but this is to be expected, as reversion occurs rather commonly, usually in the form of sectors (5).

In April, 1928, the virulence of three mutants, M–1, M–40, and M–5x, was compared with that of their respective parents. The tests were similar to those previously described (4). Mutants M–1 and M–5x possessed the same relative parasitic capabilities as they did in tests made three years previously. The virulence of the mutant M–40, however, had changed considerably. In 1925 this mutant was decidedly more virulent than its parent (4). In 1928, however, it was less virulent than its parent, form 22: in fact it was almost innocuous.

In order to test further the stability of mutants, wheat was inoculated with five of them. On reisolation four of the mutants remained unchanged in cultural characteristics and one, M–40, appeared to be slightly different after reisolation (Fig. 4). The change in this one, however, was not toward the parental characteristics. Three of these reisolated mutants were then compared as to their stability in virulence. Their sojourn on the host did not change their pathogenicity. Even the mutant M–40, which is but weakly parasitic, remained unchanged. There is no good reason of course why mutants of *H. sativum* should not mutate further in their parasitic

capabilities during their stay on living hosts. While the results in this case were negative, positive results might have been obtained if the experiment had been performed on a larger scale. These tests, however, substantiate the fact that the sectors induced by temperature are not mere ecads but true mutants.

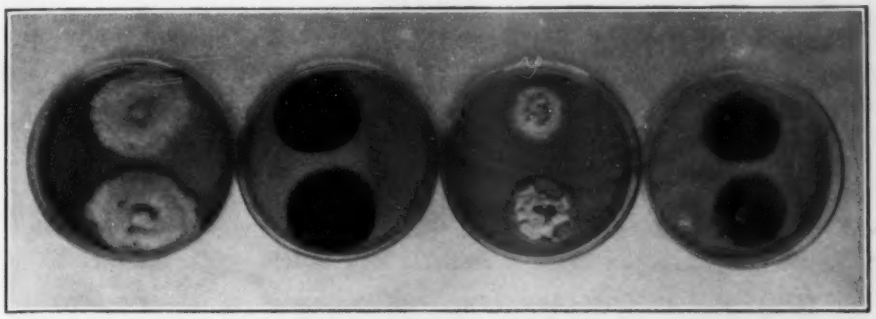

FIG. 4. Effect of passing mutants through a living host. Colonies in top row were obtained from culture reisolated from the host in April, 1928. Those in the bottom row are transfers from the original cultures. From left to right: mutants M–1, M–5xd, M–40, and M–s–27.

DISCUSSION AND CONCLUSIONS

The present studies have shown that temperature has a profound influence on the frequency of mutation in *Helminthosporium sativum* on culture media. The results indicate that moderately high temperatures induce mutation. By far the greatest number of mutants appeared at temperatures between 25° and 30° C. There was some indication that the optimum temperature for mutation in forms 9 and 12 was slightly higher than the optimum for mutation in forms 1, 10, 22, and 35. However, more work is necessary before a definite statement can be made on this point. Mutations seldom occurred at temperatures below 20° C. or higher than 30° C. Although mutations were not observed at the lower and relatively high temperatures, it is possible, but not very probable, that they did occur in masked form. It is obvious that in the study of mutation in fungi one must take into consideration the effect of temperature on the frequency of this phenomenon.

During the past four years the writer has observed hundreds of sectors, or apparent mutants. Many of them have been isolated, cultured, and studied through many clonal generations. Several of these mutants have remained strikingly different from their parents for nearly four years, but some have reverted to the parental type. In general, growing mutants on the living hosts had no apparent effect on the stability of the mutants.

The cultural characteristics of all the mutants which were passed through the living host, with one exception, remained unchanged. Their pathogenic capabilities remained stable in all these tests. These facts support the view that sectors usually are true mutants and not mere phenotypic changes.

SUMMARY

1. A study was made, under controlled conditions, of the effect of temperature on the frequency of mutation in *Helminthosporium sativum* grown on artificial media.
2. Temperature has a profound effect on the frequency of mutation. The optimum temperature for mutation in *H. sativum* lies in the vicinity of 25° to 27° C. No mutation occurred in cultures grown at 15° C., or temperatures lower than this. Comparatively speaking, only a few mutants developed in cultures kept at temperatures below 25° C., or higher than 30° C.
3. Some of the mutants of *H. sativum* are as distinct to-day as they were on the date of isolation, nearly four years ago. Some reversions have occurred.
4. The passage of mutants through living hosts, with one exception, did not appear to change the cultural characteristics of the mutants. Their pathogenicity remained constant.

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BROOMING DIEASE OF BLACK LOCUST (ROBINIA PSEUDACACIA)

CARL HARTLEY AND FERDINAND W. HAASIS

Tree diseases of the mosaic or filterable-virus type appear to be few. Among the forest trees, spike disease of sandal is the only well-known case. When as much search has been made for such diseases in the forests as has been made in the fields, we may find plenty of them. It seems worth while to put on record a disorder of black locust suggesting the virus diseases, which has been known to botanists and foresters for many years but which appears to have escaped mention in the literature.

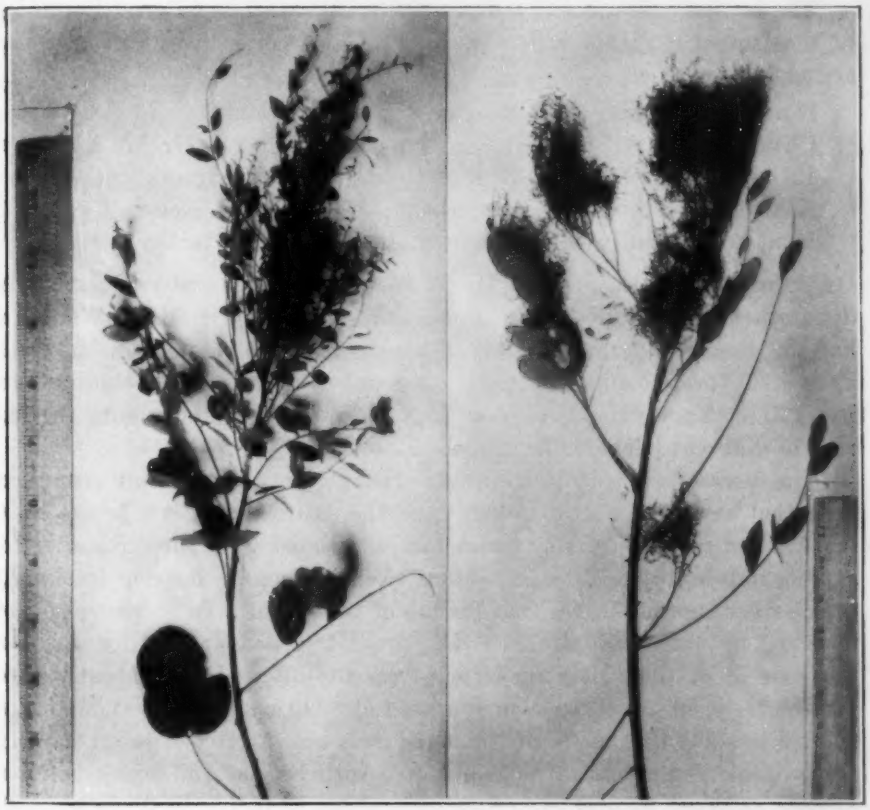

FIG. 1.—Terminal witches'-brooms on root sprouts of black locust, Asheville, N. C., September, 1926.

The characteristic witches'-brooms are shown in figure 1. There are proliferation and reduction of both shoots and leaves, and the brooms are always erect or ascending. On a single tree 7 inches in diameter at breast height, whose crown was perhaps two-thirds broomed, cushions were found on the trunk. The typical cushion is 4–5 cm. in diameter and approximately 1 cm. thick. The cushion bears groups of buds and tiny leaves on its surface, and is regarded as merely an extreme form of broom.

A number of broomed sprouts were observed and tagged on a permanent sample plot established by the Appalachian Forest Experiment Station on Curtis Creek, North Carolina, in the spring of 1922. All were dead when the plots were examined the following year. On older trees branches which bear brooms die back from the tips; the brooms themselves are also short-lived.

The earliest collections in the possession of the Office of Forest Pathology of the United States Department of Agriculture for the states indicated are as follows:

Roanoke, Virginia, August, 1908; by Horace Engler.
Chevy Chase Lake, Maryland, September 15, 1908; by Dr. W. A. Taylor.
Elijay, Georgia, August 10, 1914; by Dr. G. G. Hedgcock.
Asheville, North Carolina, August 9, 1927; by R. M. Nelson.
Mont Alto, Pennsylvania, January, 1928; by Carl Hartley.

Dr. Erwin F. Smith and Dr. Haven Metcalf independently observed the disease near Washington, D. C., some years ago, and Dr. M. B. Waite has had the disease under observation in Virginia and on his farm in Maryland since 1899 (oral communications). A number of foresters, including Professor L. J. Young and Professor E. A. Ziegler, are familiar with the disease in different parts of the Appalachians.

The disease is frequent on sprouts arising from the roots and stumps of trees that have been cut. In some cases the entire sprout is a broom, making a low, tiny-leaved plant which the uninitiated would never suspect of relationship to locust (Fig. 2). More often the brooms develop terminally or in axils of normal leaves near the top of the sprout. In at least one case, observed by Mr. G. F. Gravatt, the parent tree had appeared healthy at the time of cutting. Among larger trees the disease is apparently rare, but the frequent appearance on sprouts following cutting operations makes it seem possible that many of the older trees are infected without showing recognizable symptoms. The 7-inch tree with brooms and trunk cushions already referred to, and two other trees about 13 inches in diameter, with parts of their crowns broomed but without cushions, were found in the Chevy Chase district near Washington, D. C. The brooms are so numerous on trees that show any of them that they are obviously due to some systemic

factor rather than to local infections. Were the disease entirely limited to sprouts, it might be attributed to abnormal physiological conditions, but that seems impossible in the case of the larger affected trees which in environment and history appear identical with their normal neighbors. The

FIG. 2.—Witches'-broom of black locust arising from root of girdled tree, Woodwardville, Md., October, 1921. ×7/16. By courtesy of Dr. M. B. Waite.

Office of Forest Insects, United States Department of Agriculture, has had the disease under observation and finds no reason to suspect an insect cause, though aphids are numerous on cushions and brooms. A further study of the disease is being made by Lyle W. R. Jackson, of the Office of Forest Pathology. It is within the range of possibilities that both quantity and quality of wood produced by many apparently normal trees, and perhaps

even such characters as attractiveness to the locust borer, may be influenced by masked infections. In other parts of the world where black locust is extensively planted, it is suggested that precautions be taken against the introduction of this disease, which under other conditions might cause more damage than it does in the United States.

Systemic witches'-broom diseases are known in several other genera of legumes. Reports under the names rosette, bunching disease, and Kräuselkrankheit, indicate much damage to peanuts from disease of this type in different parts of Africa.[1] The similar krulziekte affects the same crop in Java.[2] The pale dwarf disease of peanut[3] results in plants which superficially resemble the smallest of the completely broomed locust sprouts, but are essentially different in that they show a mere reduction of parts. The leaves are small and closely bunched in the case of pale dwarf, but there is no other change in habit and no proliferation, the leaves being no more numerous than on healthy plants of equal age.

Rutgers, in his aforementioned krulziekte paper also figures an apparently systemic brooming disease of *Phaseolus mungo* in Java, and notes that similar phenomena have been reported there on *Crotalaria verrucosa* and *Dolichos biflorus*. Much proliferated terminal brooms of *Crotalaria juncea*, of unknown cause, were brought to one of the writers from Terisi, Java, by Mr. H. de Veer in 1922. He stated that 18 per cent of a planting was affected, and diseased plants produced no seed. Schwarz[4] later published photographs of brooms on *C. juncea* and *C. anagyroides* from Buitenzorg similar to those found by Mr. de Veer but none with the extreme proliferation of some of the material from Terisi. Schwarz reports that the pods failed to ripen and the immature seed germinated in the pod.

BUREAU OF PLANT INDUSTRY
AND FOREST SERVICE,
U. S. DEPARTMENT OF AGRICULTURE.

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[3] Hartley, Carl. Pale dwarf disease of peanut (*Arachis hypogaea*). Phytopath. **17**: 217–225. 1927.

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A METHOD OF INOCULATION FOR PHYMATOTRICHUM ROOT ROT INVESTIGATIONS[1]

J. J. Taubenhaus, B. F. Dana, W. N. Ezekiel, W. J. Bach, and J. P. Lusk

Root rot caused by *Phymatotrichum omnivorum* (Shear) Duggar (*Ozonium omnivorum* Shear) is a very destructive disease on many hosts. Losses to cotton alone in Texas aggregate millions of dollars yearly. Though the disease has been known for more than 30 years and investigated extensively, definite progress with it has been hindered by the nearly complete failure of experimental inoculations. Thus it was not until 1923 that the first experimental proof was furnished that *Phymatotrichum omnivorum* is the cause of root rot. King[2] inoculated cotton plants in the field by inserting cotton roots, infected naturally or from artificial cultures, in a trench dug next to the roots of the plants and then filling the trench with earth. Taubenhaus and Killough[3] succeeded in inoculating cotton plants in sterilized soil with pure cultures on mulberry stems or cotton roots. Peltier, King, and Samson[4] were successful with only a single inoculation of a number that were attempted.

With the beginning of intensive study of cotton root rot at the Texas Agricultural Experiment Station, it was realized that so long as results could be based only on observational methods, studies of even such simple matters as varietal resistance could not be conducted on a strictly experimental basis. After numerous preliminary trials, the procedure outlined below was developed by the writers and tested first on cotton plants growing in deep galvanized pots in the greenhouse. It was successful there and also in plat and field tests; and was adopted as the standard procedure in the root-rot investigations at College Station and the three substations where root-rot work is in progress. Two years of further experience with the method have proved it invaluable both when used on a wholesale scale in field work, where for instance it has made definite results possible in testing cotton strains for resistance to root rot, and when used

[1] Published with the approval of the Director as contribution No. 416 of the Texas Agricultural Experiment Station.

[2] King, C. J. Cotton root rot in Arizona. Jour. Agr. Res. 23: 525–527. 1923.

[3] Taubenhaus, J. J., and D. T. Killough. Texas root rot of cotton and methods of its control. Tex. Agr. Exp. Sta. Bul. 307. 1923.

[4] Peltier, G. L., C. J. King, and R. W. Samson. Ozonium root rot. U. S. Dept. Agr., Bur. Pl. Indus., Bul. 1417. 1926.

in experimental studies on a smaller scale, such as in determining the effect of environmental conditions on the progress of root rot. (See figure 1.)

OUTLINE OF THE METHOD

Roots of plants freshly wilted from root rot are used as inoculum. Such plants invariably have advanced root lesions, since wilting from root rot does not occur until after a perceptible, and often considerable, portion of the roots has been invaded by the causal organism. Except in very dry or extremely wet ground, these root lesions are covered with a copious surface growth of the fungus. This type of inoculum is most virulent, and a higher percentage of infection is secured with it than with roots of plants that have been wilted for a longer time. Roots that are completely dead have failed to cause infection.

FIG. 1.—Cotton plants artificially inoculated with cotton root rot. Inoculum placed at left end of box nearly three months before the photograph was made.

When cotton is used, which is usually the most readily obtained inoculum, plants apparently recently wilted are pulled by hand, the tops cut off at the surface of the ground, and the roots dropped into moist sacks. Since root rot occurs in large spots and is characterized by readily recognized symptoms of wilting and the still more characteristic mycelial strands on the roots, large amounts of diseased roots can be collected with comparative ease. These roots are kept moist and used as rapidly as possible for inoculation. The inoculum, if fresh, may be successfully shipped by mail or express, or it may be kept for a few days without great loss of virulence.

Under field conditions a crowbar is used to pierce a hole in the soil 1–1½ in. from the stem of the plant to be inoculated. One or more of the fresh pieces of inoculum are placed in this hole and the soil pressed together again. The inoculum in place should lie close to the host root, and an inch

or two below the surface of the ground. In this position the infected zone on the inoculum parallels the tap-root and is usually at some point in actual contact with some of the lateral roots.

Most successful inoculations have been secured when the moisture content of the soil is high. Under non-irrigated conditions, water has been supplied locally about the plants as soon as inoculum has been inserted. A thorough soaking has given best results.

This method has proved particularly useful with crews of three men, when inoculating large plats. One man goes ahead opening holes with the crowbar, followed by a second man who inserts the inoculum, and a third who covers the holes and applies the water.

RESULTS WITH THE METHOD

Mass inoculations in the field are frequently made with one piece of inoculum inserted near the base of each cotton plant. The first results above ground appear in from ten days to two weeks, with the wilting and subsequent death of an occasional plant. In three to four weeks, 40–80 per cent of the plants are usually killed. This may be considered the result of initial, rapid infection. Following this initial killing there is almost always further infection, usually causing the death of most of the remaining plants in the next four weeks. This final loss may be indirect, as the result of spread of the disease from plants which succumbed first, or it may be merely from delayed original infection.

Thus of 840 cotton plants at College Station, Texas, inoculated early in the summer of 1928 with the roots of infected cotton plants, 346, or about 41 per cent had wilted in the next 24 days. Of 52 cotton plants inoculated with carrots that were naturally infected with root rot, 26 had succumbed to the disease in six weeks. But in both these lots, almost every plant was killed by root rot within the following three weeks, while there was still less than 1 per cent of the disease in adjoining check rows.

In making inoculations on a smaller scale for greenhouse or plat work, higher initial infections are secured by using a larger amount of inoculum for each plant and watering more liberally. For instance, cotton plants grown for soil-type studies in a series of 40 wooden containers were inoculated by this method but with four pieces of inoculum for each container. Periodically, these plants were washed out and examined. Within a week of the inoculation, abundant mycelial growth was visible along the tap-roots; after another week these portions of the tap-roots were superficially decayed; and it was only after this that many of the plants began to show any injury above ground. The inoculation proved to be successful in 39 of the 40 containers.

Although root rot is able to attack the majority of cultivated crops, yet no cross-inoculations have hitherto been reported. By the use of the inoculation method described, the writers have already made successful cross-inoculations from cotton to cowpeas, tomatoes, alfalfa, guar, lima beans, eggplant, peach and prune trees. Likewise, cotton, sugar beets, cowpeas, soy-beans, sunflowers, pear and apple trees, and grape vines have been successfully inoculated with carrot inoculum. Sweet potatoes and beets have been used to infect cotton; and the roots of a diseased jujube tree have infected a mulberry tree and cotton.

DIVISION OF PLANT PATHOLOGY AND PHYSIOLOGY,
TEXAS AGRICULTURAL EXPERIMENT STATION.

PHYTOPATHOLOGICAL NOTES

A New Cotton Wilt[1]—Early in the fall of 1927, our attention was called to a disease which was said to cause heavy damage to cotton in Ellis County, Texas. Some plants were examined and found to be affected by a wilt which appeared different from the common cotton wilt caused by *Fusarium vasinfectum* Atk. During 1928, the disease has been found prevalent in Ellis County, in heavy clay soils and in fields in which root rot [*Phymatotrichum omnivorum* (Shear) Duggar] was also prevalent. We have recently found what appears to be the same disease in El Paso County and Brazos County. These three counties are in widely separated sections of the state, so that a wide distribution at least in Texas is indicated.

Superficial symptoms of the disease resemble those of the common Fusarium wilt. The plants are decidedly stunted, especially if infection occurs early in the season; and are peculiarly branched with abnormally short, stout joints. With the advance of the disease the plant sheds its leaves and the branches become dull in color. In some cases, plants die from the tops, and new growth appears on the lower part of the main stem. Only a small percentage of the affected plants succeed in making a boll or two of cotton. Occasionally, infected plants are so dwarfed and stunted as to take on the appearance of a rosette.

The new disease may be distinguished from Fusarium wilt by splitting the stems. In both diseases there is blackening; but in the case of the new wilt the discolored area is confined mostly to the interior cylinder of both roots and stems, while with typical Fusarium wilt it is mostly in the outer, woody tissue. (See Fig. 1.) In the new disease, the discoloration is more pronounced in the lower part of the plant and is progressively less higher on the stem.

About 400 isolations have been made from roots and stems of affected plants from a field in Ellis County in which there was 60 per cent loss from the disease, and parallel cultures made from typical Fusarium wilt of cotton from Athens in east Texas, where this disease is so prevalent. A pure culture of *Fusarium vasinfectum* was obtained in practically every case from either roots or stems of the cotton from Athens. On the other hand, many organisms were isolated from the Ellis County plants. One of these is a *Fusarium* which has from the beginning appeared distinct from *F. vasinfectum*. The other organisms isolated are species of *Alternaria, Sclerotinia, Phoma, Phomopsis,* and *Helminthosporium*. Work is now in progress to determine the pathogenicity of any or all of these organisms.

[1] Published with the approval of the Director as contribution No. 417 of the Texas Agricultural Experiment Station.

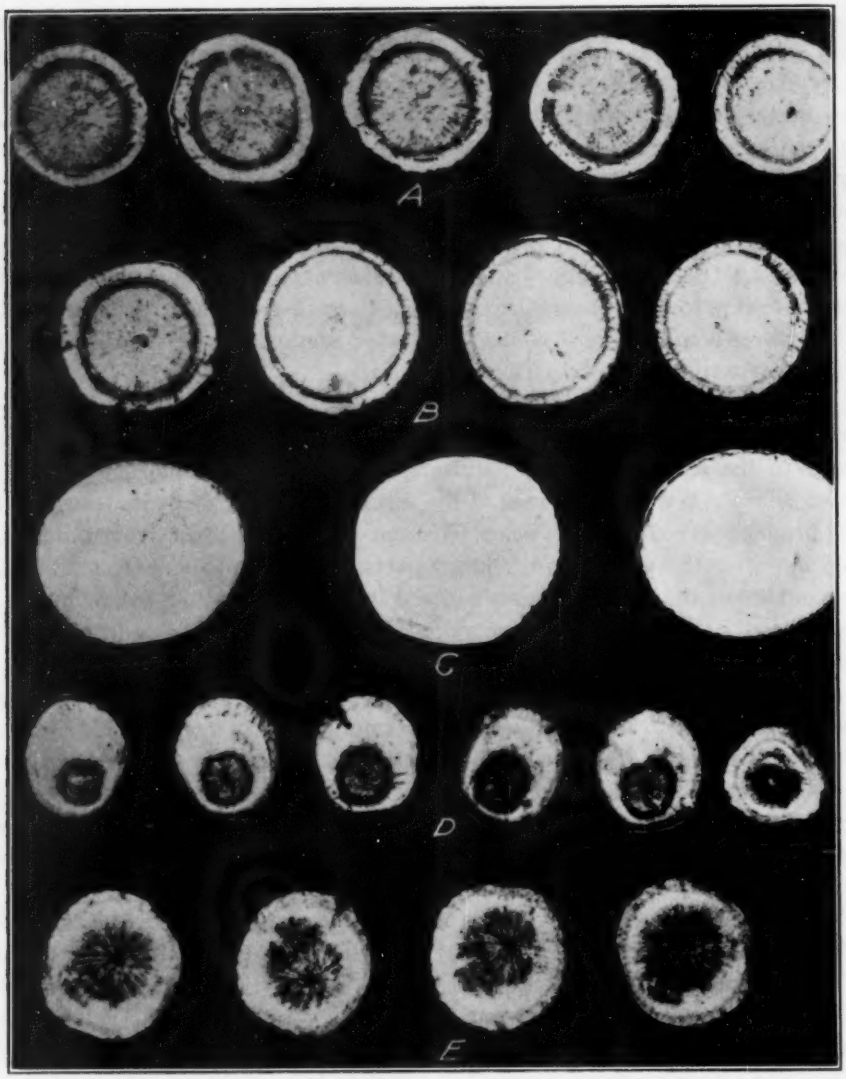

FIG. 1. Cross-sections of cotton roots and stems. A and B, the wilt caused by *Fusarium vasinfectum*. C, sections of normal plants for comparison. D and E, the new cotton wilt.

It is of particular significance that this disease has been found in the same soils and in the same fields as those in which root rot is destructive. Workers with the old Fusarium wilt of cotton in the United States have long considered this disease as peculiar to light, sandy soils. Some of us[2] have

[2] Taubenhaus, J. J., Walter N. Ezekiel, and D. J. Killough. Relation of cotton root rot and Fusarium wilt to the acidity and alkalinity of the soil. Texas Agr. Exp. Sta. Bul. 389. 1928.

recently shown that this relation is apparently a matter of soil acidity. Fusarium wilt appears to be confined mostly to soils more acid than pH 6.5 —7.0. On the other hand, Phymatotrichum root rot seems to be destructive only on soils that are more alkaline than this. The two diseases are, in fact, never found in destructive abundance in the same parts of the same field; and the Fusarium wilt has not been of considerable importance in the regions in which root rot has been abundant. In this new disease we are thus confronted for the first time with a destructive cotton wilt in the heavy black lands.

Search through the American literature failed to reval any description of the disease in question. Fahmy,[3] however, makes brief mention of an apparently similar cotton disease which he found to occur in Upper Egypt. He describes a peculiar discoloration in resistant varieties such as the Ashmouni or Zagora varieties which was very different from the common Fusarium wilt, and refers to this as "Upper Egypt" discoloration for the reason that it was observed in Upper Egypt where the Ashmouni and Zagora varieties are grown. This discoloration was localized in a part of the central cylinder of the root, hypocotyl, or lower part of the stem. Isolations made by Fahmy from these discolored portions yielded a *Fusarium* which failed to infect the Ashmouni or Zagora varieties and which he accordingly considered non-parasitic. It is regrettable that Fahmy's figure of the "Upper Egypt" discoloration is not very distinct. From his description, however, the Texas disease is much the same as that found in Upper Egypt. Should this prove true we could not agree with Fahmy that the discoloration in the roots is of no consequence, at least not under our conditions. In Texas, this interior discoloration of the central cylinder of the roots and stems of cotton is associated with a definite and serious wilt disease of the plants.—J. J. TAUBENHAUS, W. N. EZEKIEL, and H. E. REA, Texas Agricultural Experiment Station, College Station, Texas.

Dusting seed for oat smuts.—Until the recent work was done in Ohio with formaldehyde and iodine dispersed in infusorial earth,[1] no satisfactory dusts had been developed for controlling the two smuts of oats in spite of the fact that extension men and growers have been asking for a dust treatment comparable to the one for stinking smut of wheat. An opportunity to compare these Ohio dusts with some organic mercury dusts under New York conditions presented itself in the spring of 1928.

[3] Fahmy, Tewfik. The Fusarium disease of cotton (wilt) and its control. Min. Agr. Egypt, Tech. and Sci. Service Bul. 74. 1928.

[1] Sayre, J. D., and R. C. Thomas. Formaldehyde and iodine dusts for the control of oat smut. Ohio Agr. Exp. Sta. Bimonthly Bul. 13: 19–21. 1928.

The results of this experiment were so striking and apparently so significant that a short note regarding them seems justified. Of course no sweeping conclusions can be drawn from a single experiment, but the results of this one are nevertheless of interest. A supply of glumed oat seed naturally infected was obtained through the courtesy of Dr. W. H. Tisdale of the E. I. Du Pont de Nemours and Company. Thirteen lots of seed placed in separate mason jars were well shaken with the different dusts at the rate of 3 ounces to the bushel. A fourteenth lot was left untreated as a check.

The chemicals used in the experiment are as follows: KlC, KlB and 68, all organic mercury compounds from E. I. Du Pont de Nemours and Company, Wilmington, Delaware; 200, 197, 196, 195, 194, 193, 192, and 189, also organic mercury compounds, from the Agricultural Department of the Bayer Company, Inc., New York City.[2] Iodine, 1 per cent in infusorial earth, and Smuttox which is 4 per cent formaldehyde in infusorial earth were obtained from Grasselli Chemical Company, Cleveland, Ohio.

TABLE 1.—*Mean percentage of oat smut in six replicated rod rows for each dust treatment*

No.	Treatment	Smut in per cent		
		Total	Loose smut	Covered smut
1	Check	[c]12.62 ± 0.06	[a]3.33	[a]10.2
2	DuPont KlC	0.11 ± 0.01	0.00	0.11
3	DuPont KlB	0.33 ± 0.009	0.11	0.22
4	DuPont 68	3.22 ± 0.06	[a]0.93	[a]2.20
5	Grasselli Iodine	13.11 ± 0.69	[b]0.40	[b]9.92
6	Grasselli Smuttox	0.33 ± 0.01	[a]0.00	[a]0.27
7	Bayer 200	1.67 ± 0.03	[a]0.13	[a]1.40
8	Bayer 197	4.33 ± 0.04	0.78	3.56
9	Bayer 196	2.67 ± 0.04	0.61	2.06
10	Bayer 195	3.44 ± 0.03	[a]1.00	[a]2.47
11	Bayer 194	3.44 ± 0.04	0.50	2.94
12	Bayer 193	2.06 ± 0.03	0.28	1.78
13	Bayer 192	3.17 ± 0.04	[a]0.47	[a]2.81
14	Bayer 189	7.67 ± 0.07	[a]1.53	[a]6.00

[a] Only 1500 panicles in the series.

[b] Only 1200 panicles in the series.

[c] Discrepancies between total smut and the sum of the individual smut species occur because the two smuts were not counted separately in one or more rows.

[2] The fungicide, insecticide, and disinfectant departments of these two companies have consolidated under a new subsidiary, the Bayer-Semesan Company, Inc., since these materials were procured.

Seed of each treatment were hand planted in rod rows replicated 6 times in soil which was well prepared. The hands were carefully cleaned between each sample. After the oats had headed, 100 contiguous panicles were counted in 3 different places in each rod row making a total of 1800 panicles for each treatment and 2000 panicles for the check, because 200 additional heads were counted in the last check row of the series. Except in one or two cases the two smuts were counted separately. The experimental results are set forth in table 1.

The facts brought out by the data are: (a) that Du Pont dusts KlC and KlB[3] and Grasselli Smuttox controlled both smuts very satisfactorily; (b) that Bayer dust 200 is slightly less effective, while Bayer nos. 197, 196, 195, 194, 193, and 192 are intermediate, and Bayer 189 is ineffective; (c) that Du Pont 68 is in a class with most of the Bayer dusts in this experiment; (d) that the iodine dust was inefficient under the conditions of the Ithaca experiments.—JAMES G. HORSFALL, Cornell University, Ithaca, New York.

A new and striking abnormality in potato plants.—An abnormal condition in potatoes, believed to have been undescribed hitherto, was observed during the summer of 1928 in a single crop of Rural Russet potatoes on Vancouver Island, British Columbia, Canada.

The most striking features in all plants obviously affected was the presence, below the surface of the ground, of a tumor-like tuber on every stem. The stem, arising from the set, entered this swelling at its base and, emerging from the apex, continued on to the light and air to bear leaves. Although the tumor bears eyes, it is not a normal tuber simply penetrated by the stem, but is an unusual growth of the stem tissues.

Other symptoms apparently associated with this peculiar development are a dwarfed plant and fewer and smaller tubers, most of which are malformed. No symptoms such as mottling or wrinkling of the leaves were noted, but the dwarfed and immature condition of the aerial part of the plant was sufficiently definite to enable one to select severely affected plants in the field with ease. The vines in such cases would be about 3 inches in height and quite green, as compared with those of normal plants approximately 16 inches high and approaching maturity. Between these two extremes there was a gradation, and here the separation of normal from abnormal plants was more difficult without examining the stem below the ground for the characteristic growth. In all cases, however, plants exhibiting the tumor of the stem were noticeably dwarfed.

[3] Since these experiments were performed, the Bayer-Semesan Company has placed on the market a dust intermediate between KlC and KlB, under the trade name, "Ceresan."

Approximately 1 per cent of the plants in the crop showed this abnormality, and the grower states that the same condition has been present to a greater extent in past years.

As the problem is being investigated with a view to ascertaining whether it is a further addition to the list of virus diseases affecting the potato, any related information or material would be greatly appreciated by the author.—L. M. BLACK, Department of Botany, The University of British Columbia, Vancouver, B. C., Canada.

Cultures of sclerotial fungi.—During the past 15 years I have been accumulating cultures of sclerotial fungi of all kinds, in connection with studies on the taxonomy of *Sclerotinia* and *Botrytis*. The total number of strains which we have had in culture to date approximate 1600. While some of these have died, we still have growing in stock approximately 1000 strains. Under a special grant from the Heckscher Foundation for Research in Cornell University, we are able to maintain these cultures year after year. Many workers throughout the United States and in other parts of the world have, from time to time, made many contributions of specimens and cultures of sclerotial fungi for our use.

We should greatly appreciate it if all workers who may have occasion to collect or isolate sclerotial fungi of any kind would send us cultures or freshly collected specimens, preferably both, whenever they come to hand. We are glad to make any of our cultures available to other workers in so far as is consistent with our obligations to contributors and the progress of our own investigations, believing that in this way we will be rendering service to our colleagues at very little inconvenience to ourselves.

We are particularly anxious to get cultures of any newly described species whether they be of *Botrytis*, *Sclerotinia* or other sclerotial fungi. Workers on any of these forms need have no hesitancy in sending such cultures or forms on which they may be working, as we shall be very glad to maintain them in culture under such restrictions as to distribution or use by us in our investigations as the sender desires to make. Full data as to time, host, place of collection, etc., should accompany the sendings.

Hearty coöperation is invited of all workers or collectors of these forms in this effort to maintain a large and representative culture collection of sclerotial fungi.—H. H. WHETZEL, Cornell University, Ithaca, New York.

BOOK REVIEW

Principles of Plant Pathology. By Charles Elmer Owens. Edition I, 929 pages, 222 figures. 1928. John Wiley and Sons, Inc.

Although a number of excellent treatises on different phases of plant pathology have been published in recent years, their nature has been such as to require the student to purchase several books in order to gain a comprehensive view of the subject. It is gratifying, therefore, to receive the new book by Owens, entitled "Principles of Plant Pathology." As the title indicates, the author presents the principles of the science in a way that appears to be especially usable in teaching undergraduate students.

Part I of the book consists of a discussion of topics of general interest concerning the nature, cause, and control of plant diseases, comprising an interesting review of the history of plant pathology and its place relative to other branches of science. This part is unique in furnishing the student with material on which to base a broad view of plant pathology.

Chapters are devoted to such subjects as symptoms of plant diseases, and their classification, methods of investigating plant diseases, and the effect of external factors on plant diseases. Control measures are discussed particularly in chapters on fungicides, disease resistance, and quarantine. The relation of insects to plant disease and problems of storage, transportation, and marketing are also treated.

Part II presents specific diseases selected from a wide field representing types of causal agents and types of symptoms. The history and distribution of the plants attacked, and symptoms and economic importance of diseases are discussed. This discussion is followed by a description of the organisms, their life cycles, and control measures. At the end of each chapter a valuable supplementary list of diseases is given together with selected references, also directions for laboratory work, and a list of review questions. This sort of discussion is helpful both to the student and to the instructor.

While the general scheme of Part II is based on a mycological classification of the causal agents, this is subservient to the presentation of types and basic principles. The bibliographies following the chapters are well selected and the book is profusely illustrated with excellent photographs and line drawings.

The subject matter on plant pathology has become so voluminous that in the average course it is hopeless to try to present more than the leading and essential facts. Dr. Owens' book serves this purpose well since its outstanding feature is its general treatment of the subject, bringing to the student the important points of plant pathology. This is well summed up in the title "Principles of Plant Pathology." I believe the use of this book will prove it a worthy text.

L. W. Durrell, Colorado Agricultural College.